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Stem Cells Transplantation and Aerobic Exercise: A Therapeutic Combination in an Animal Model of Parkinson’s Disease

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Stem Cell Transplantation and Aerobic Exercise: A Therapeutical Combination in an Animal Model of Parkinson’s Disease

Dissertation / Thesis submitted to postgraduate program in Rehabilitation Sciences of the Federal University of Health Sciences of Porto Alegre as a requirement for the obtaining the degree of Master.

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DEDICATION

There are a number of people that without their support and constant motivation, this project would not have been successfully developed and I have a special feeling of gratitude with them.

To my parents, Sandra Hurtado and Juan David Cucarián owing their permanent reinforcement that I have taken as an example of encouragement and inspiration to me throughout my life.

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It is so unbelievable how powerful and strong are the experiences we lived when we need to live them.
RESUMO

O tratamento terapêutico ideal para os sintomas motores da doença de Parkinson (PD) ainda não foi desenvolvido, no entanto, o transplante de células tronco surgiu como uma abordagem promissora e revolucionária. Apesar dos resultados positivos de estudos experimentais e de alguns ensaios clínicos permanece insatisfatório, não só para mitigar completamente os distúrbios motores, mas também é insuficiente para contribuir com a recuperação funcional. Portanto, o objetivo deste estudo foi investigar o potencial terapêutico das células tronco mesenquimais humanas derivadas de tecido adiposo (ADhMSCs) em combinação com um programa de exercício físico (PE) em um modelo de DP por 6-hidroxidopamina. Quarenta e um ratos machos Wistar foram divididos em cinco grupos de tratamento (sham, lesão, células, exercício e combinado). hMSCs foram isoladas e transplantadas estereotáxicamente no estriado com uma taxa de administração de $2 \times 10^5$ células em 6 μL, além disso, os roedores que compuseram os grupos exercício e combinado foram submetidos a um treinamento progressivo aeróbico em esteira. Subsequentemente, a avaliação neurocomportamental foi desenvolvida pelo Foot-Fault Task. Melhoria significativa na função motora e na posicionamento das extremidades foram relativamente semelhantes em todos os grupos de tratamento. Porém, o exercício e o grupo combinado foram os melhores na recuperação funcional. Em contraste, foi identificada uma deterioração permanente nas funções locomotoras nos ratos que não receberam nenhum tipo de tratamento. A avaliação da imunofluorescência foi conduzida para detectar neurônios positivos à tirosina hidroxilase (TH) na substância nigra pars compacta (SNc). Nesse sentido, a presença de neurônios TH-positivos foi corroborada visualmente nos grupos de tratamento em comparação com a baixa concentração no grupo lesão. Em conjunto, nossos dados sugerem que ADhMSCs e PE são adequadas estratégias de abordagem para proporcionar efeitos terapêuticos na recuperação motora em ratos com DP, não obstante devem ser desenvolvidas mais pesquisas que estudem os efeitos e os mecanismos terapêuticos destas estratégias em combinação, melhorando o entendimento da associação destas no processo da neuro-recuperação deste transtorno degenerativo.

Palavras-chave: Doença de Parkinson; Terapia celular; Células tronco; regeneração; Exercício físico; Neuroplasticidade; Capacidade neurotrófica.
ABSTRACT

The ideal therapeutic treatment for Parkinson's disease (PD) motor symptoms has not been developed yet, nevertheless, the stem cell transplantation had emerged as a promising and revolutionary approach. Despite the positive results of experimental studies and some clinical trials, remains been unsatisfactory not only to mitigate completely the motor disturbances but also is insufficient to contribute in the functional recovery. Therefore, the aim of this study was to investigate the therapeutic potential of adipose-derived human mesenchymal stem cells (ADhMSCs) combined it with physical exercise (PE) in a model animal of PD by 6-hydroxydopamine. Forty one wistar male rats were divided into five groups of treatment (sham, injury, cells, exercise and combined). hMSCs were isolated and transplanted stereotactically into the striatum at a rate of administration of \( 2 \times 10^5 \) cells in 6 \( \mu \)L, in addition, the rodents that composed exercise and combined groups were submitted an aerobic progressive treadmill training. Consequently, the neurobehavioral assessment was developed by Foot-Fault Task. Significant improves on motor function and paws placing were relatively similar in all treatment groups. Nonetheless, the exercise and combined groups were better in the functional recovery. In contrast, was identified a permanent deterioration in the locomotor functions in rodents that not received treatment. Immunofluorescence evaluation was conducted to detect tyrosine hydroxylase (TH) antibodies in the substantia nigra pars compacta (SNC). In this sense, the presence of TH-positive neurons was visually corroborated in the treatment groups in comparison low concentration within the lesion group. Taking together, our data suggest that ADhMSCs and PE are good approaches to provide therapeutic effects in the motor recovery in rats with PD, further research in the combined these strategies should be developed, improving the understanding of the mechanisms that could be involved in the neuro recovery of this disorder.

**Key words:** Parkinson's Disease; Cell-based therapy; human mesenchymal stem cells; regeneration; Physical Exercise; Neuroplasticity; Neurotrophic Capacity.
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<tr>
<td>6-OHDA</td>
<td>6-Hydroxydopamine</td>
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<tr>
<td>ADhMSCs</td>
<td>Adipose-Derived human Mesenchymal Stem Cells</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-Brain Barrier</td>
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<tr>
<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
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<tr>
<td>bFGF</td>
<td>basic Fibroblast Growth Factor</td>
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<td>BG</td>
<td>Basal Ganglia</td>
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<td>BGTC</td>
<td>BG-ThalamoCortical</td>
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<td>BM-MSC</td>
<td>Bone Marrow-Derived Mesenchymal Stem Cells</td>
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<tr>
<td>BrdU</td>
<td>Bromodeoxyuridine.</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>CPG</td>
<td>Central Patterns Generator</td>
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<tr>
<td>CRPC</td>
<td>C- Reactive Protein</td>
</tr>
<tr>
<td>CTC</td>
<td>Cerebellar dentate-ThalamoCortical</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Medium</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine Tetraacetic Acid</td>
</tr>
<tr>
<td>ESCs</td>
<td>Embryonic Stem Cells</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
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<tr>
<td>FGF</td>
<td>Fibroblast Growth Factor</td>
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<tr>
<td>FOG</td>
<td>Freezing of Gait</td>
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<td>FSG</td>
<td>Festinating Gait</td>
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<tr>
<td>GDNF</td>
<td>Glial cell line-Derived Neurotrophic Factor</td>
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<tr>
<td>I.P</td>
<td>Intraperitoneally</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon-Gamma</td>
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<td>IGF-1</td>
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<td>IL-6</td>
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<tr>
<td>iNOS</td>
<td>inducible Nitric Oxide Synthase</td>
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<tr>
<td>iPSCs</td>
<td>induced Pluripotent Stem Cells</td>
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<tr>
<td>LB</td>
<td>Lewy bodies</td>
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</table>
**LN**  Lewy Neuritis

**MAS**  Multiple System Atrophy

**MFB**  Middle Forebrain Bundle

**MLR**  Mesencephalic Locomotor Region

**mNSCs**  murine Neural Stem Cells

**MPTP**  1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

**MSC**  Mesenchymal Stem Cells

**NF-κ B**  Nuclear Factor kappa-light-chain-enhancer of activated B cells

**NGF**  Nerve Growth Factor

**NO**  Nitric Oxide

**NPCs**  Human Neural Progenitor Cells

**NSAIDs**  Nonsteroidal Anti-Inflammatory Drugs

**NSCs**  Human neural stem cells

**NT-3**  Neurotrophin-3

**OS**  Oxidative Stress

**PA**  Physical Activity

**PBS**  Phosphate-Buffered Saline

**PD**  Parkinson Disease

**PE**  Physical Exercise

**PFA**  Paraformaldehyde

**PGC-1α**  Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

**ROS**  Reactive Oxygen Species

**SCs**  Stem Cells

**SDF-1α**  Stromal Derived Factor-1 alpha

**Shh**  Sonic hedgehog

**SMA**  Supplementary Motor Area

**SNc**  Subtantia Nigra Pars Compacta

**TH**  Tyrosine Hydroxylase

**TNF-α**  Tumor Necrosis Factor Alpha

**tPA**  tissue Plasminogen Activator

**VEGF**  Vascular Endothelial Growth Factor
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INTRODUCTION

Individuals who suffer from Parkinson Disease (PD) are severely affected in their quality of life-related with health; this situation is reflected in the increasing levels of physical and cognitive disability as a result of this neurodegenerative condition, becoming a critical scenario that requires effective and adequate therapeutic approaches.

As a result, it is not weird that this pathology is considered as a devastating disorder affecting on annual average between 10-20 subjects per 100,000 inhabitants in the worldwide (Tysnes & Storstein, 2017), being the second most prevalent degenerative disease. As a consequence is to be expected that patients and caregivers may have the need to incur high costs in the healthcare systems, this social reality has been widely reported (Kowal, Dall, Chakrabarti, Storm, & Jain, 2013).

Scientific comprehension and the recent advances in the perception of the mechanisms related to this disorder have recognized the complex of physiopathological processes that are implicated and contribute to the motor and cognitive impairments that overlapping and disturb the functionality, interpersonal interaction and restrict the social participation of patients. In this sense, the etiology hallmarks of this illness that have described include the depletion of dopamine (DA) neurons in the Sustancia Nigra pars compacta (SNc) and the striated nucleus, the aggregation of Lewy neurites and bodies, increases in the level of oxidative stress, mutations in α-synuclein and parkin genes and lastly, but not least, some environmental factors are also implicated in the onset of this condition (Lotharius & Brundin, 2002).

Despite the advances in the understanding of the complex etiology of this disorder and the development of interdisciplinary treatment modalities, the efforts are not sufficient and the majority of these therapeutic approaches focus on the symptoms, being currently a challenge the restraint in the disease progression. Stem cells (SCs) transplant have emerged as a promising and revolutionary method to treat the hallmark symptoms of PD, providing a cellular source to restitute dopaminergic pathways and enhance the neuro-microenvironment. In spite of positive findings from animals studies and some clinical trials in the motion performance due to the reinstatement of circuits and DA levels, and
the improvement in cellular environmental conditions, increasing thus, the rate survival of the remaining dopaminergic neurons, this therapeutic approach is not enough for mitigating absolutely all the symptoms. From this notion, we considered that is possible increase the benefits of the SCs transplant combining it with an effective, economical and proven treatment in patients with PD as is the case of physical exercise (PE). In this sense, was developed a literature review about the pathophysiological mechanisms involved in PD and the evidence from human and animals experimental research in the field of cellular therapies to treat this disorder. In addition were described the effects of PE in the modify of disease progression. In order to corroborate our hypotheses was conducted an experimental study, testing the effect of human derived-adipose tissue mesenchymal stem cells (ADhMSCs) and an aerobic progressive training in modeling rats of PD by 6 hydroxydopamine (6-OHDA). The results of this project were showed and described in an empirical report.
OBJECTIVES

Main Objective:

To determine the potential effectiveness of the synergic combination of human mesenchymal stem cell transplants derived from adipose tissue and the aerobic physical exercise as an adjuvant method in the treatment of locomotor performance in an animal model of PD.

Specific Objectives:

1. Recognize the degree of the dopaminergic lesion through the methylphenidate test and immune-staining techniques.

2. To identify the effectiveness of the Foot-Fault Task in the as a sensible test of motor decline in rats models of PD.

3. To assess the degree of functional recovery among the experimental groups through the rotational behavior in and the motor coordination the in the Foot-Fault Task.
LITERATURE REVIEW

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc) and the inclusion of Lewy bodies (LB). This condition is typified by the overlap of the motor and non-motor symptoms, being clinically disabling and challenging to treat (Schapira et al., 2014). Currently, research efforts have focused to determine and elucidate the pathophysiological mechanisms involved. Cellular and animals models have revealed impairments on the oxidative stress, dysregulation in the neuroinflammation process and mitochondrial dysfunction [1]. In addition, these advances have contributed to developing novel therapeutic strategies that not only deal with the symptoms but also attempt to improve the state of the diseased neuron-glia networks, decreasing the rate of cellular degeneration, therefore, its progression.

Cell therapy has been a boom in the last decades to replace/repair the degenerated cells and modify positively the cellular microenvironment through multiple cellular mechanisms that mediate the recovery process, including the release of multiple trophic factors, the modulation of cytokines pro or anti-inflammatory and the processing of discard elements. In a similar way, Physical Exercise (PE) is a well-recognized and used as an adjuvant approach that improves physical health and contributes to the enhancing of functional capacity of people with chronic and neurodegenerative diseases (Ang et al., 2010; Lunn et al., 2011). Emerging evidence based on animal studies and some clinical humans trials suggests that these both therapeutic strategies are related with the improve of motor performance, being a relevant resource that could be potentiated in conjunction strengthening, the benefits already obtained from these approaches per se

In general, it has been reported that these approaches promote neurogenesis, have a protective function on the remaining survival neurons and contribute to the restorative effect on dopaminergic neurons [4–6]. Nevertheless, the use of a combined therapy of cellular transplantation and exercise to PD is scarcely studied. Is for that reason that in this review, we provide an update on recent progress and the potential effectiveness in the use of Stem cell (SCs) transplantation and PE for the treat Parkinson disease, based on scientific evidence in clinical trials and animal studies.
Parkinson's disease: Economic Burden and Etiology Factors

PD is a complex neurodegenerative long-hold movement disorder that affects thousands of people, it is characterized by important impairments in the motor control and proprioceptive system. Nowadays, it is well known that non-motor deficits are included in the hallmark symptomatology of this illness, leading to a devastating impact on the functionality and a high rate of disability (Ziemssen and Reichmann, 2007). It is considered the second most prevalent pathology in the worldwide, after Alzheimer disease, affecting often people between 40-50 years, being the masculine gender the more prevalent 2:1. Its social, psychological and health burden are heavy, therefore, generating an important disruption in the productive life and the quality of the normal aging process (Elbaz et al., 2016). The advanced ages are major risk factors, only in industrialized countries, 1% of people 60 years of age or above have this condition. In consequence, not surprising that its incidence is around 8 to 18 per 100,000 person-years based on population prospective studies [5]. In this context, the financial scenery is a relevant issue in the social-health context, due not only for hospitalizations, pharmacologic therapies, outpatient visits, home care and other necessary requirements but also for indirect costs for patients and caregivers associated with loss of productivity, early retirements, personal health costs and copayment treatments (Soundy et al., 2014). Only in the United States, the expenses for this population was approximately $14 billion in 2010. In the same way, the cost that patients and caregivers have to assume is not cheap being estimated to be close to $10,000 per person, an amount that is expected to increase in the following decades (Kowal et al., 2013). This perspective is close to European countries, where was demonstrated a directly proportional relationship between the progression of the pathology and rise of expenses (Lindgren et al., 2005). Unfortunately, in Latin America is unknown with certainty the real economic impact of this disease due to the lack of adequate socio-demographic studies and official reports.

As well as in the majority of neurodegenerative diseases, the etiology in PD is multicausal, involving wide interrelations of factors that have not been fully established. These elements are composed of modifiable characteristics (e.g. environmental exposures and lifestyles) and nonmodifiable factors (genetic elements). However, the real cause is even
unknown. As already mentioned is clear that the process of aging is a relevant factor in the development it; nevertheless, it does not cause it on their own. Some factors have been identified and might have an influence on the onset of this neurological condition including: lower education rates, traumatic brain injury, hypertension and the occupational profile (Elbaz et al., 2016).

**Molecular and Cellular Mechanisms of Pathogenesis of PD.**

The loss of dopaminergic neurons in the SNc is a relevant factor associated with PD symptomatology, a loss between 70%-80% of this neurons is required before the symptoms begin to be recognizable (Smith, 2008), a fact that indicates that the degeneration process begins many years before the pathological features manifest. However, other mechanisms also contribute to cell death; some include mitochondrial dysfunction, oxidative damage, genetic heterogeneity and metabolic alterations (Reeve et al., 2014) that result in an inadequate system of detoxification and energetic use.

Neuronal inclusions of α-synuclein in the perikarya and cell processes referred as LB (Lewy bodies) and Lewy neurites (LN), respectively, are one of the most common etiological disturbances described in PD. Apparently, the localization of these proteins inclusion are associated with the progress of neurodegeneration evident in this condition. From this perspective, people with mild to moderate clinical symptoms have more presence of LB-LN in lower regions of the brainstem, in contrast, it has been identified greater placement in rostral brain areas in chronic cases. These intraneuronal proteinaceous inclusions are also linked with a multiple system atrophy (MSA) disorder, where an evident progressive impairment of nigrostriatal dopaminergic and cerebellar afferents pathways occur (Abeliovich and Gitler, 2016a; Dickson, 2012; Ferrer and Isidro, 2011). In this sense, both pathological processes contribute to support the prion hypothesis, where the progression of the disease is directly related to the distribution from one brain area to another through the presence of αsyn, as a relevant factor that determinate the severity of the PD (Visanji et al., 2013).

In addition to the presence and deficits in the protein synthesis systems, recent studies also have identified pathological changes on the morphology and performance of
mitochondria in patients with PD. It has been suggested that it would promote the
destruction of dopaminergic neurons, contributing to the onset and progression of this
condition through different mechanisms that are not now well established, but which
clearly take part in the functional deterioration. (Esteves et al., 2008; Keeney et al., 2006;
Moon and Paek, 2015). Based on this causal relation the presence of reactive oxygen
species (ROS), the decrease in the mitochondrial complex I enzyme activity, the ATP
depletion and the caspase 3 activation leading to the augment on oxidative stress (OS)
in the nigral neurons aid to the cell death and clinical progress of symptoms (Niedzielska
et al., 2016).

Another particular process that characterizes PD is the neuroinflammation, determined
by the chronic release of cytokines and pro-inflammatory factors mediated by the
activation of astrocytes and microglia altogether with T cell infiltration, these are the
principal attributes detected in both patients and animals models (Wang et al., 2015) and
would be associated with the depletion in dopaminergic neurons. Pro-inflammatory
mediators, including, tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ) and
interleukin-1β (IL-1β) are highly expressed. In addition, the activation of the phenotype
M1 of microglia is a particular feature detected in many neurodegenerative conditions and
PD is not an exception; authors have related it with the increase in the release of cytotoxic
molecules such as the nitric oxide (NO) and inducible nitric oxide synthase (iNOS), and
the cyclooxygenase-2 (Kones, 2010). That is how this chronic neuroinflammatory process
partakes in the constitution of ROS and free radicals (Qian et al., 2010) promoting the
nerve cell degeneration, dopaminergic toxicity, and cell apoptosis, being an important
target to take into account in the development of effective therapeutic strategies.

Although this neurodegenerative condition is considered a sporadic disorder, is now clear
that the emergence of a proportion of cases (5%-10%) are caused by genetic mutations,
in this context, alterations in genes as PARK2, PARK6, PARK7, and PARK8 have been
associated with this phenotype of parkinsonism, being more frequently studied the
genetic implications in it onset.(Abeliovich and Gitler, 2016b; Kones, 2010). In summarize,
is clear that the etiology of PD is multifactorial, the genetics, environmental factors, and
life styles have a relevant role in the vulnerability of humans to suffer this devastating
disorder. Nonetheless, despite the advances of research in this field, is imperative to go
ahead in the understanding of the interrelation of these etiologic mechanisms. This way, effective methods for treating motor symptoms and to halt the disease progression can be developed.

**Motor and Non-Motor Clinical Features of PD**

The Parkinsonism is a permanent and progressive syndrome characterized by resting tremor, muscle rigidity, bradykinesia, postural instability and coordination disturbance, being these the cardinal symptoms of this neurological condition. Their onset is often unilateral (asymmetrical) and gradually affect the opposite body hemisphere (Williams and Litvan, 2013).

One of the typical and most confusing clinical signs is the rest tremor. This impairment involves, in acute stages, the distal regions of the upper and lower extremities; while in chronic stages, the proximal joints are compromised and the tremor in action (reemergent tremor) begins to appear. It is established that the severity of tremor is not related to the degree of degeneration of dopaminergic neurons in the SNc; recent studies suggested that this impairment is associated with deficits of other dopaminergic core, the retrorubral nucleus (Helmich et al., 2012), and the cerebellum. The relevance of cerebellum in the modulation of tremor was identified from experimental studies that confirm that cerebellar stimulation could induce modifications in the timing of peripheral tremor, being the cerebellar dentate-thalamocortical (CTC) and BG-thalamocortical (BGTC) the targeting key circuits in the control of tremor amplitude (Helmich et al., 2011; Hirschmann et al., 2013). Thalamus, basal ganglia (BG) and premotor regions are cortical structures that have been implicated with this clinical sign, but is not yet well demonstrated their mechanisms of action and their relevance in the progression of this functional impairment (Duval et al., 2016).

Similarly, a classical disruption evident also in PD patients is the bradykinesia, which consists of a reduction in the development, coordination, speed, and amplitude of corporeal movements. The neuro-mechanisms implicated are not well established and understood; nevertheless, their onset is correlated with an imbalance between the direct and indirect circuits between BG and motor cortices. Regarding this, it has been
demonstrated a superior activation of indirect pathway, leading to decrease in the level of excitation within the Thalamo-cortical networks. As well the hypoactivation of pre-motor, supplementary motor (SMA) and somatosensory areas are associated with the disbalance among dopaminergic circuits, generating aberrant sensorimotor integration, which is reflected in low rates of muscle voluntary contraction and proprioception disturbances (Berardelli et al., 2001; Cano-de-la-Cuerda et al., 2010; Espay et al., 2009).

The rigidity is a secondary and relevant element implicated in the decline of motion (Magrinelli et al., 2016). It is characterized by an increase in the muscle tone and the resistance to passive movement, being the flexor pattern the prevalent in these patients. Abnormalities in peripheral sensory inputs and pathological changes in mechanical components of joints (tendons-muscles) have been established as responsible mechanisms in the increment of discharge of motoneurons. The intensification in neuronal activity contributes to the latency of the stretch reflex and the maintenance of this pathological posture (Delwaide et al., 1991; Magrinelli et al., 2016; Rothwell et al., 1983). In this sense, it is important to emphasize that PD patients also present somatic, sensory, vestibular and visual perception abnormalities, key components that contribute to increase the postural instability and the risk of fall. Likewise, musculoskeletal disturbances including, the decrease in the magnitude of the force, velocity and muscle resistance take part in the postural impairment and lead to gait deficits. In this respect, many mechanisms have been involved in the gait disturbances, including pathological changes in the function of SMA, primary sensory-motor cortex and cerebellum. These pathological adaptations are related to the onset of freezing of gait (FOG) and the festinating gait (FSG), common motor experiences in this type of patients (Balash et al., 2012; Chen et al., 2013). Equally, new evidence point to the main role of the mesencephalic locomotor region (MLR) in the control of gait and balance. This structure, particularly, possesses several reciprocal interconnections with the cerebellum, cortical areas, and BG, that once interrupted cause deficits in the initiation, maintaining and modulation of the gait (Jahn et al., 2008; Magrinelli et al., 2016). Finally, the decline in the connection with motor neurons in the spinal cord (central patterns generator, CPG), DA dysfunction, disruptions in different neurotransmitters, including the noradrenergic, serotoninergic, and cholinergic systems are as well other important protagonists in the
onset of gait disorders (Dietz, 2003; Magrinelli et al., 2016; Takakusaki, 2017). Besides these distinctive motor symptoms, non-motor complaints are also prevalent. Systems participating in the regulation of mood, cognition and memory, sleep–wake cycle control, sensory perception and autonomic balance are also disrupted to different levels (Bonnet et al., 2012; Ziemssen and Reichmann, 2007). The subsequent manifested symptoms overlap with the motor complaints, hampering the effectiveness of pharmacological or therapeutic treatments. In many cases, they are underrecognized and undertreated, being a real challenge in advanced stages of PD.

**Biological Mechanisms of Action of Stem Cells Therapy in the Repair and Protection of Brain Tissue**

Stem cells (SCs) therapies are a revolutionary and promising therapeutic approach in the clinical and research areas. They have been used for the treatment of numerous pathologies and neurodegenerative conditions are not an exception. The SCs are a type of cell that has self-renewal capacity and can differentiate into multiple lineages of cells; these properties make them an important clinical alternative in the treatment of patients with chronic conditions. Fundamentally, cellular therapies use tissue or cells grafts to treat diseases for two therapeutic objectives: the replacement of cells and the enrichment of the cellular environment (Lunn et al., 2011). Transplanted cells could differentiate, integrate and be part of new neuro-glial functional networks within the host and secrete a diversity of neurotrophic factors that could optimize the environment that supports host neurons aiding to cellular recovery. These features have been deeply studied. In this order of ideas, the presence of growth factors including glial-derived (GDNF), vascular endothelial (VEGF) and brain-derived neurotrophic factors (BDNF) after transplantation have been related to relevant for neuroprotective and restorative functions in the nervous system (Lunn et al., 2011; Suzuki and Svendsen, 2008; Wu et al., 2016). What is more, a decrease of the pathological inflammatory process, restoration of neurotransmission, stimulation of plastic responses in the host have been also linked with a functional re-establishment of afferent-efferent connectivity (Sanberg et al., 2012). Additionally, processes of neovascularization through the production of angiogenic factors (e.g. VEGF) (Lindvall et al., 2004) could boost the endogenous recovery
processes by means of an increase of anti-inflammatory molecules and the reduction of OS levels and apoptosis pathways. Horie et al. reported that after sub-acute transplantation into the ischemic human brain, stem cells secreted VEGF that induced vascular repair and decreased the inflammatory reaction (Horie et al., 2011a). Similarly, an improvement in the blood-brain barrier (BBB) integrity by up-regulating tight-junction protein expression has been reported (Horie et al., 2011b).

One of the most studied components in the regenerative area using SCs include the modulation of anti-inflammatory response, it is characterized by a suppression of the peripheral adaptive immune response, the depletion in brain infiltration of immune cells, and the decreased brain edema (Pluchino et al., 2008). In this sense, cellular transplantation have been demonstrated to reduce the expression of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κ B) in neurodegenerative conditions (Kim et al., 2013).

Is clear that neurodegenerative disorders, as well as other neurological conditions, modify the morphology and performance of neurons and glial cells. SCs can potentially revert this by enhancing neural connectivity, synaptogenesis, remapping processes and augment the expression of markers related to neuroplasticity (Lu et al., 2012). These effects were corroborated in studies in animal models of spinal cord injury where was described that SCs-grafted promoted cellular adaptations that up-regulate axonal outgrowth, rewiring, and sprouting (Lu et al., 2012). Similar effects were found in rats with stroke where an increment in the branching, length, and arborization of dendrites was observed after human neural progenitor cells (NPCs) transplant, that contribute to the functional improvement (Horie et al., 2015).

An additional molecular effect recently identified is the enhancement in the endogenous Sonic hedgehog (Shh) pathway; this glycoprotein participates in many physiological processes, including embryonic development, cellular repair, and brain plasticity. Ding et al. showed that SCs therapy increased synapse reorganization, oligodendrogenesis, and axonal density by up-regulating of this pathway and the endogenous tissue plasminogen activator (tPA) (Ding et al., 2013). In the same line, Ager et al showed morphological adaptations after transplantation of murine neural stem cells (mNSCs) in the hippocampus of mice with neuronal loss. In this case, an increase in the synaptogenesis
in striatum radiatum of the CA1 intrahippocampal region was evidenced that was correlated with enhancement in cognitive function through the release of proteins like synaptophysin and synapsin (markers of synaptic integrity) (Ager et al., 2015).

It is well recognized that adult neurogenesis is a dynamic physiological process that occurs in specific cortical areas, the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone of the lateral ventricle in the striatum. The plastic nature of the brain is attributed to the presence of this cycle. This regular process aids to the formation of functional new neurons that establish connections within the existent circuitry and that is involved in synaptic plasticity and better information processing (Kempermann et al., 2015). In the neurodegenerative conditions, this capacity is disturbed, which allows the degenerative process. Fortunately, cell transplantation therapies have demonstrated increase neurogenesis due to the stimulation of growth factors and the release of chemokines such as Notch receptor ligands, BDNF, NGF and stromal derived factor-1 alpha (SDF-1α) that promote plastic and regenerative responses in the host (Horie et al., 2015; Kim et al., 2013; Lindvall et al., 2004; Nicaise et al., 2015). These elements are essential for stimulate or normalize endogenous neurogenesis, contributing to the ameliorating of symptoms. Nevertheless, there is yet limited understanding of the mechanisms whereby the transplanted cell-induce or contribute to this physiological process.

Lastly, it is important to emphasize that the therapeutic objective of SCs therapy depends on the pathologic process and its effectiveness will be influenced by many factors including host responsiveness, the type and number of transplanted cells and the employed technique.

**Stem Cell-based Therapy for Parkinson’s disease.**

Promising advances in the use of stem cells to treat neurodegenerative conditions have made this therapeutic approach an attractive alternative for treating symptoms in PD. The major research challenge for developing a therapeutic strategy for this neurological condition consists in establishing an efficient and effective method to slow down, or stop it altogether, the progression of the disease.
In PD, the substitution of dopaminergic cells has been the typical purpose for SCs therapy. Thus far, this approach is apparently attainable. However, in recent years, the additional mechanisms have been also mattering of inquiry. Numerous cells sources have been used for treating PD, including bone marrow-derived mesenchymal stem cells (BM-MSC), adipose-derived mesenchymal stem cells (AdMSCs), human neural stem cells (NSCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), being the three last ones the most promising for induction into dopaminergic neurons with similar morphological structure and electrophysiological properties (Fig.1.) (Feng and Gao, 2012; Li, 2012).

**Fig.1** Different tissue resource for the treatment of PD. Recent advances have proportioned wide range options for obtaining neural progenitor cells (NPCs) and cellular sources for treatment schemes. (A) Embryonic Stem Cells (ESCs) High capacity of self-renewing and proliferation, provide many different types of cells from the three germ layers. (B) Mesenchymal Stem Cells (MSCs) Multipotent cells with an immunomodulation property to reduce immune responses in the host. Despite this, these cells have limited ability to differentiate into dopaminergic neurons. (C) Neural stem cells (NSCs): Isolation from the fetal brains, blastocyst or induced pluripotent stem cells (iPSCs), are multipotent and would differentiate in the own cells of the nervous system. Low risk of tumor formation. Is difficult their preservation and rate of proliferation for long periods of time. (D) iPSCS. Pluripotent allogeneic cells that are reprogrammed to an undifferentiated state. Are obtained from the own individual minimizing the risk of rejection. This type of cell has some potential to form tumors.
In this light, it is also remarkable that transplanted SCs differentiate into glial cells, being a fundamental factor in the enhancement of the microenvironment and functional recovery in patients and animal models with PD. Commonly animals models of nigrostriatal dysfunction have been induced by 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and their construct, face and predictable validity are widely recognized and demonstrated in research (Mercanti et al., 2012). The use of mesenchymal stem cells (MSCs) transplants in these models have brought about a decrease in the neuronal loss (Glavaski-Joksimovic and Bohn, 2013; Joyce et al., 2010; Kitada and Dezawa, 2012). In addition, the increase of secreted neurotrophic factors including NGF, BDNF, fibroblast growth factor (FGF) and the upregulation of tyrosine hydroxylase (TH) are other potential benefits induced by this type of cells (JIN et al., 2008). These molecular effects are functionally correlated with a reduction in the motor behavioral effects of the dopaminergic lesion and partial restoration of nigrostriatal circuits (Han et al., 2015). Some authors considering that the promotion of endogenous neuronal growth factors, the regulation of neuroinflammatory factors, the decreased of apoptosis and the improvement in cerebral microenvironment identified in animal models are strong evidence that suggests the possibility of finding similar physiological results in humans from this therapeutic approach (Marks et al., 2008; Mittermeyer et al., 2012; Venkataramana et al., 2010). Despite the beneficial implications of this type of cell in the recovery of neurogenerative disorders, a potential disadvantage is that it is easily susceptibility to the underlying pathology environment. For instance, MSCs grafted in rats with Alzheimer disease showed the presence of β-amyloid peptide, contributing thus with the neurodegeneration (Joyce et al., 2010).

NSCs are another important cell type for transplantation, these cells can easily differentiate into neurons and glial cells (astrocytes and oligodendrocytes). In severely impaired primates by MPTP, an improvement in motor performance and an increased expression of growth factors were identified after they were grafted. The authors related this functional recovery to the neuroprotective, angiogenic and neurogenic effects induced by these type of cells (Cattaneo et al., 2014; Gincberg et al., 2012; Nishino et al., 2000; Sanberg, 2007). Some few clinical trials have been conducted and positive results
in motor behavior and histological assessments had been also noticed (Defer et al., 1996; Freed et al., 2001; Lindvall et al., 1994; Piccini et al., 1999). Finally, the use of ESCs and iPS cells have demonstrated a greater functional integration, higher graft-induced production of DA and ultimately, the better improvements in the motor behavior of monkeys and rats as models of this pathology (Fu et al., 2015; Zhang et al., 2016). Despite this, some limitations are evident, in the case of ESCs includes their poor survival rate, the high risk of developing teratoma and ethical dilemmas. On the other hand, iPS have emerged as an excellent and promising alternative to use the own patient cells providing a good option to reprogramming and differentiated it, minimizing thus the graft rejection and the avoiding the complicated immunosuppression procedures (Han et al., 2015).

The compile of findings here reported having demonstrated that the cell replacement therapy is a useful source for treating PD and other neurodegenerative conditions. Notwithstanding, the exact mechanisms through which they could foster beneficial effects remain largely unknown. This area of research is exciting from the clinical and neuroscience prospects; it is clear that there is a long way yet to develop a standard and safe procedure to use this method. Despite this, scientific research has done great progress in developing techniques for isolating, differentiating and grafting SCs. This achievement is visible from the positive results of clinical trials and models animals with neurological impairments that support the feasibility of this therapeutic approach in the treatment of degenerative disorders.

*Physical Exercise as a Protective Factor against Neurodegenerative Conditions.*

The PE in one of the most prescribed therapies in health and disease. It has positive effects on the physical and cognitive well-being and reduces the relative risk of chronic diseases related to aging. Thus, through it, it is possible to enhance the quality of life of normal aging process and reduce early mortality. Similarly, there is strong evidence supporting the preventive nature of PE in several neurological pathologies, including neurodegenerative conditions (Ang et al., 2010; Voelcker-Rehage and Niemann, 2013).
Enhancements in motor and cognitive functions are common results of physical training, being widely documented the neuro-protective effects involved in functional improvement. Increased cerebral blood flow, activation of cortical areas and improved neuroplasticity are thought to mediate it (Hayes et al., 2013; Voelcker-Rehage et al., 2011). Within the later, increases in the synaptic and dendritic density, angiogenesis, rise in the number of glial processes and hippocampal volume has been described (Gomez-Pinilla and Hillman, 2013a; Voelcker-Rehage and Niemann, 2013).

Studies in rodents identified that after a program of voluntary exercise the capillary density and the number of synapses in the Purkinje cells increased within the cerebellum. Similar vascular outcomes were described within the motor cortex and the hippocampus, where the rate of new neurons (neurogenesis) rose after a physical activity (PA) program (Pereira et al., 2007; Thomas et al., 2012). Research in animals models with neurodegenerative conditions had demonstrated that exercise promotes the neuroplasticity and recovery process by up-regulating the number of new neurons and strengthening of neuronal connections (Svensson et al., 2015; Voss et al., 2013). In the same way, empirical studies in elderly people demonstrate that the exercise and PA also increase the gray matter volume in the prefrontal cortex, the temporal lobe, and the hippocampus (Erickson et al., 2014; Voelcker-Rehage and Niemann, 2013). This structural molding within the brain has been associated with improvements in cognitive processing, movement speed, balance, fine coordination, visuospatial and motor processing. In contrast, few studies have assessed the effects in the volume of white matter. So far, positive changes in its volume in older adults after 6 months of cardiovascular training have been seen. This adaptation, apparently, is related to a better processing speed but not improved cognitive performance (Jacobs et al., 2013).

Neuroprotective and anti-inflammatory factors are induced, including the release of BDNF, VEGF, and IGF-1 that promote the up-regulation of neurogenesis, angiogenesis and contribute to the neurovascular adaptations (Gomez-Pinilla and Hillman, 2013b). Equally, an anti-apoptotic effect, namely, a decline in neuronal death and inhibition of hippocampal apoptosis in some traumatic and inflammatory conditions have been reported (Jee et al., 2012; Jung et al., 2014).
Different modalities of exercise have demonstrated positive effects in health conditions in humans, being the aerobic training the more studied. Improvements in working memory, cognitive flexibility and attention are the principal results obtained from this style of physical conditioning (Baker et al., 2010; Gomez-Pinilla and Hillman, 2013a). Furthermore, aerobic training leads to anti-inflammatory effect due to the release of IL10 (a potent anti-inflammatory factor) and the reduction of pro-inflammatory interleukins such as IL1β, IL6 the TNFα, and the C-reactive protein (CRP) (Aguiar et al., 2011; Petersen and Pedersen, 2005). On the other hand, the resistance training also has shown positive effects related to the increased the release of IGF-1, that promotes neurogenesis in the hippocampus, and the myelination; adaptations that have been related to enhanced memory and less cortical white matter atrophy in older women (Best et al., 2015). Cotman et al reported that this type of training has also neuroprotective effects associated with activation of immunomodulatory mechanisms, the delivery of trophic factors and the regulation of ROS and OS (Cotman et al., 2007). All together these findings are reflected in a improve in selective attention, solving problems, and associative memory after physical conditioning programs based on strength training (Liu-Ambrose et al., 2012; Nagamatsu et al., 2012).

These positive findings are also extrapolated to patients with neurodegenerative diseases. Exercise has been shown to reduce the progression of the Alzheimer disease by aiding in the “cleaning” of the amyloid-beta peptide (Ahlskog et al., 2011). In addition, and similarly to cellular therapy, PA enhances the production of cerebral growth factors that attenuate cholinergic neuronal death and the cognitive decline (Chen et al., 2016). The reduction in OS, the increase in the energy metabolism and the cerebral blood are positive effects of this approach that upgrade the neurogenesis and the micro-environment, being substantial elements that counter the neurodegeneration and are linked with the regular practice of exercise (Chen et al., 2016).

*Neuroprotective Benefits of Physical Exercise in Parkinson Disease.*

Patients with PD have also been benefited from this therapeutic method. Aerobic exercise has been shown to decrease the progression of the disease and improve postural
stability, balance, gait pattern and muscular coordination (Ahlskog, 2011; Lauzé et al., 2016). Furthermore, growth factors and hormones releasing might play a relevant role in promoting endogenous neurogenesis (Ang et al., 2010). The prevention of cognitive decline, the regulation of mood and the modulation of neuroinflammation process are positive effects related with this therapeutic approach (Cruise et al., 2011; Nocera et al., 2010). Similar effects have been described in models animals; learning and memory were benefited from an increased neurogenesis, neuroplasticity, and substantiated release of neurotrophic factors (e.g. BDNF). This factor has also seen to improve angiogenesis, DA levels and the expression of dopaminergic receptors minimizing the dopaminergic dysfunction (Erickson et al., 2011; Petzinger et al., 2010; Robertson et al., 2016; Speelman et al., 2011; Vučković et al., 2010; Wu et al., 2011). Likewise, it has been detected partial reconstruction of nigro-basal ganglia circuits and a GABAergic modulation that contribute with the recovered of the cortico-subcortical connectivity, corticomotor excitability and therefore the motor performance (Borrione et al., 2014; Fisher et al., 2008; Shin et al., 2017; Yin et al., 2009). Additionally, PE activates endogenous anti-oxidant systems and down-regulated the expression of glutamate receptors implicated in excitotoxicity. This discovery was derived from an experimental study in an animal model of PD, where the intensive exercise restored the glutamate receptor expression, boosting the capture of this neurotransmitter and lowering its extracellular concentration (VanLeeuwen et al., 2009).

Strength training has also brought benefits in gait pattern, coordination, motor learning, muscle resistance and balance in clinical trials (Cheng et al., 2016; Tambosco et al., 2014). Few studies have gone deep into the molecular mechanisms linked to these benefits. Some of them include the release of Insulin-like growth factor-I (IGF-I), that per se promotes VEGF and BDNF secretion, higher vascular density, and improved glucose consumption (Frazzitta et al., 2013; Rojas Vega et al., 2010). Furthermore, resistance training constantly practiced reduces the rate of pro-inflammatory basal cytokines (of IL-6 and TNF-α) (Calle and Fernandez, 2010; de Salles et al., 2010) and increases that of anti-inflammatory cytokine (IL-10) (Schwenkgrub et al., 2013).

The evidence and data here exposed supports the premise that PE, independently of their modality (aerobic or resistance training), is a fundamental tool for promoting
neuroplasticity and neuroprotection, two properties associated with motor and cognitive recovery in animal models and patients with PD.

*Combined Therapy: Stem Cells Transplantation and Physical Exercise for Neurodegenerative Conditions.*

Despite the wide positive effects reported from these two therapeutic approaches in the treatment of motor symptoms and progress of PD that have been summarized here, based on our knowledge, it does not exist research that have assessed the therapeutic potential in the combined of these strategies in animal models or patients with PD. Similarly, scarce studies have used this conjunction to treat neurodegenerative conditions. In the case of patients with amyotrophic lateral sclerosis, their use demonstrated an improvement in the functional independence and the prolongation in the life expectancy in contrast with physical training per se (A.A et al., 2015). The use of exercise as an adjuvant to the cellular therapy in cases of spinal cord injury in rodents and humans has promotes motor recovery, through the reconstitution of pathways conduction and the stimulation of CPG (Ichim et al., 2010; Tashiro et al., 2016). In addition was identified a better glial scar formation from this combined scheme of treatment, promoting a better reconstruction of synaptic circuits (Nicola et al., 2016). Notwithstanding, in spite of the positive results in some neurological conditions, there are also studies that had not identified a superior functional recovery through the combination of these strategies (Nicola et al., 2016; Wang et al., n.d.). Thus is clear the presence of a gap in the understanding of the interaction of these therapeutic methods and the activation of physiological processes that support neuro-recovery. Parameters such as the type of training, the intensity of exercise and the therapeutical recovery window have been postulated as fundamental and responsible factors that have to be defined to optimize the result for a combinatorial treatment (Fu et al., 2016). Nevertheless, more research must be conducted to supply an answer about the effectiveness of combined therapy in the treatment of neurodegenerative diseases.
Conclusion

SCs transplantation and PE are two therapeutic modalities that have been associated with neuroprotective and restorative properties in health and disease. With proven functional benefits in the motor and cognitive domains, both strategies are promising therapeutic approaches for neurodegenerative conditions such as PD, being both potentially disease-modifiers. What is of great interest is the fact that both strategies share, to a great extent, their so far proven cellular and molecular mechanisms of action. This overlap might open the door for a therapeutic potentiation when both therapies are used in conjunction. However, this has been poorly explored to this day, and therefore remains a fundamental gap that deserves further inquiry. Anyways, from this review, we can say that both therapeutic approaches have demonstrated to be beneficial and mitigate to different degrees the progression of the disease, improving physical and functional capacity in both patients and animals models of PD.
References


ORIGINAL ARTICLE-

Physical Exercise and Human Adipose-Derived Mesenchymal Stem Cells Ameliorate Motor disturbances in a Rat Model of Parkinson’s disease.

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Abbreviations List.

PD: Parkinson Disease; MSC: Mesenchymal Stem Cells; PE: Physical Exercise; SCs: Stem Cells; ADhMSCs: Adipose-Derived human Mesenchymal Stem Cells; 6-OHDA: 6-Hydroxydopamine; TH: Tyrosine Hydroxylase; SNc: Substantia Nigra Pars Compacta; BG: Basal Ganglia; LB: Lewy Bodies; SNC: Substantia Nigra pars compacta; PA: Physical Activity; I.P: Intraperitoneally; MFB: Middle Forebrain Bundle; PBS: Phosphate-
Buffered Saline; **DMEM**: Dulbecco’s Modified Eagle’s Medium; **FBS**: Fetal Bovine Serum; **EDTA**: Ethylenediamine Tetraacetic Acid; **PFA**: Paraformaldehyde; **BSA**: Bovine Serum Albumin; **BDNF**: Brain-Derived Neurotrophic Factor; **NT-3**: Neurotrophin-3; **bFGF**: basic Fibroblast Growth Factor; **GDNF**: Glial cell line-Derived Neurotrophic Factor; **NGF**: Nerve Growth Factor; **VEGF**: Vascular Endothelial Growth Factor; **DA**: Dopamine; **IGF-1**: Insulin-like growth factor 1; **PGC-1α**: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; **MPTP**: 1,2,3,6-tetrahydropyridine. **hMSCs**: human Mesenchymal Stem Cells

**Abstract**

Parkinson’s disease (PD) is a disabling, devastating and the second most common neurodegenerative condition. This situation may become even worse due to is expected that its prevalence increases in the worldwide in the next few years (Findley, 2007).

For decades, the motor symptoms and the overlapping of cognitive impairments make a challenge the treatment of this condition that despite the advances in the research and medical treatments, their management remains in the symptomatic therapy, being unsatisfactory, expensive and suboptimal. In this sense, is a greatly needed to develop new neurorestorative and neuroprotective therapies aimed to ameliorate motor deficits and contain the progression of this pathology. The growing evidence from experimental studies in animal models of PD and some clinical trials confirm the use of the mesenchymal stem cells (MSCs) transplant and physical exercise (PE), separately, as potent tools to treat degenerative diseases. Being then, the exercise a strong adjuvant alternative that would optimize and strengthen the effects of the cell therapy in the treatment of neurodegenerative conditions. Therefore, this study aimed to elucidate the therapeutic potential role of a combined therapy of adipose-derived human mesenchymal stem cells (ADhMSCs) and an aerobic progressive training program of exercise in the motor performance of the PD rat model induced by 6-Hydroxydopamine (6-OHDA). In this study, 41 male Wistar rats were categorized into five groups: sham, injury (6-OHDA), 6-OHDA+exercise, 6-OHDA+cells, and 6-OHDA+combined. After to assess of the
dopaminergic depletion and its extension in the methylphenidate test in our rats. The cellular resource following isolation and culture was transplanted into the left striatum of Parkinsonian rats with a lesion in the middle forebrain bundle. Two groups also were submitted to a forced run on a treadmill during four weeks (5 days/week, 30 up to 60 min/day, a speed of 16 m/min). As behavioral evaluations, Footfault Task was performed at 8 weeks after inducing the model with a consequent euthanasia for immunohistochemical investigations. All therapeutic strategies exhibited a significant decreasing in the induction of rotations by Foot-Fault Task. Similarly, limbs coordination and the paws placing on the behavioral test were improved compared to the non-treatment sample. The enhancement in locomotor performance was more evident in the 6-OHDA+exercise and 6-OHDA+combined groups. Overall, the results of this study confirm that exercise is a powerful option to improve motor performance and reduce dopaminergic lesion. Additionally, this research encourages the necessity to develop more studies in this field using the exercise as an adjuvant therapeutic approach to optimize the benefits of MSCs transplantation in the treatment of motor symptoms in PD, improving thus, the understanding of the reciprocal interrelationship between these therapeutic approaches and the mechanisms involved in the neuro-recovery of this motor disorder.

Keywords

Parkinson’s Disease; Cell-based therapy; mesenchymal stem cells; regeneration; Physical Exercise; Disease-Modifying Therapies; Neuroplasticity; Neurotrophic Capacity.
1. Introduction

Parkinson Disease (PD) is the second most chronic and progressive neurodegenerative pathology after Alzheimer disease, with an incidence between 5 and 10/100,000 person-year that usually affected more men than women (Elbaz et al., 2016; Pringsheim et al., 2014). It is a common motor movement disorder characterized by an onset unilateral with progressive motor symptoms including bradykinesia, imbalance, rigidity and rest tremor, being factors that disturb functionality and incur in high levels of early disability. Recently has been demonstrated a clinical multisystemic phenotype that involves non-motor symptoms such as depression, apathy, and cognitive impairments that contribute to the severity of symptoms, the disease progress and worsening of the quality of life (Poewe, 2008). In consequence, the clinical management includes a wide range of treatments that reflected in important expenses for patients and caregivers. Only in the United States the population with PD incurred in medical cost around $14 billion of dollars in 2010, this economic burden is projected to grow in the next few years (Kowal et al., 2013).

Deficits in the connection of pathways between basal ganglia (BG), cerebellum and cortical areas, accumulation of α-synuclein in the soma and neuritic processes (Lewy bodies (LB)), decrease in the motor and premotor cortical excitability, mitochondrial dysfunctions and loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) are pathophysiological mechanisms that characterize this degenerative disorder (Magrinelli et al., 2016). In this context, the current treatments are focused on the disabling motor symptoms, being unsatisfactory to restrain the progression of this neurological condition.

The stem cells (SCs)-based therapy has been proposed as a promising therapeutic approach to endorsing functional recovery in the nervous tissue damage and prevent the progress of the disease. This attractive strategy of treatment is based on the capacity to re-establish functions in the nervous tissue, through the release of neurotrophic factors that have demonstrated to be useful in the promotion of environmental enrichment and cell regeneration. Additionally, their faculties including the capacity to migrate and integrate neuroglial pre-existing circuits, the cell restitution and the prevention in the output of neurotoxic components that support and have been correlated with
improvement in motor and cognitive performance in animal studies and some clinical trials (Bouchez et al., 2008; Freed et al., 2011; Fu et al., 2015; Moon et al., 2013; Yang et al., 2008).

In this sense, one of the most beneficial and studied therapeutic approach to prevent and reduce motor and cognitive decline associated with degenerative diseases is the physical exercise (PE). Cumulative evidence has indicated its relevant role in the neurorecovery process and the potential benefits obtained from your regular practice, including the increase in the neuronal branching, angiogenesis, release of neurotrophic factors and boosting neurogenesis process. (Ang et al., 2010). Altogether, these benefits are translated into protective action against cerebral damage and a resource that enhance physiological bioactivity, being some of the positive effects of this effective and low-cost strategy (Itoh et al., 2011; Morgan et al., 2015). In this regard, the cells transplant and the use of exercise as a concomitant strategy would bring even better results in motor performance, that their use in an independent way. To confirm this assumption, we studied the effects of a combined treatment using adipose-derived human mesenchymal stem cells (ADhMSCs) and aerobic progressive exercise training in the motor performance in an animal model of Parkinson disease by 6-hydroxydopamine(6-OHDA) assessed by the Foot Fault task. A challenge that has demonstrated a high sensibility in the analysis of locomotor activity in PD rodent models (Silvestrin et al., 2009).

2. Materials and methods

2.1 Animals

Male Wistar rats between 200-250 gr were obtained from Breeding Unit of Federal University of Health Sciences of Porto Alegre (UFCSPA). The animals were housed three per cage with access to food and water ad libitum, with standard laboratory conditions in a 12:12 hr light/dark cycle (lights off at 17:00) and steady temperature (22 ± 2 °C). All experimental procedures were developed in accordance with the Conselho Nacional de Controle de Experimentação Animal (CONCEA) and the local Ethics and Research Committee (UFCSPA, protocol N0176/15) approved this research.
2.2 **Experimental Groups**

Rats were randomly distributed in accordance with the modality of treatment into five groups: sham-operated group (Sham; n=9), unilateral 6-OHDA injury group (Injury; n=8), progressive aerobic exercise group (6-OHDA+Exercise; n=8), hMSCs grafting group (6-OHDA+Cells; n=8) and hMSCs transplant and aerobic exercise (6-OHDA+Combined; n=8).

![Experimental Timeline](image)

**Fig. 1.** Experimental Timeline. All rodents got through all the phases of this study. Sham received a NaCl lesion/graft within the same stereotaxic coordinates but not was exposed to any treatment. Similarly, the injured group did not take part of any therapeutical intervention. All samples underwent identical behavioral tests at the same time points.

2.3 **Middle Forebrain Bundle Infusion of 6-OHDA**

The animals were anesthetized with Xylazine (10mg/kg) intraperitoneally (I.P) and Isoflurane between 4-5% with oxygen administration into 1-1.5 L/min, to maintaining the level of anesthesia. The concentration of Isoflurane was administered among 2%. To avoid and control pain was injected tramadol I.P in the onset of the surgical procedure. The body temperature was maintained in normothermia (37 °C) using a heating pad. They were positioned in the stereotaxic frame (Kopf Instruments, C.A., USA) and the scalp was partially removed, 12 µg 6-OHDA (Sigma, Aldrich) was used to produce a dopaminergic neuronal loss, it dissolves in a solution of sterile saline (0.9%) containing ascorbic acid (Sigma, Aldrich 0.1%). The injection rate was 1 µL/min using a 10 µL microsyringe (Hamilton 701 N, Sigma, St. Louis, M.O., USA) and an infusion pump (11 Plus Harvard Apparatus, W.A., USA). The cannula was placed during 3 minutes to prevent a reflux in the left middle forebrain bundle (MFB) with coordinates anterior-posterior (AP)
-4.0mm, medial-lateral (ML) 1.3 mm and dorsal-ventral (DV)-7 mm relative from bregma and dura (Torres et al., 2011) according to the rat brain atlas.

Lastly, was used iodopovidone to disinfection the region of surgery and subcutaneously injection of sterile saline to hydrate. Rats received post-operate care until awake. For the sham-operated group, the same surgical procedure was developed and an equivalent saline solution volume was injected. The animals were supervised and allowed to recover for 3 days.

2.4 Methylphenidate-induced Rotational Behavior.

To corroborate the dopaminergic damage, two weeks after surgery procedure, the turning response of animals was assessment through the Methylphenidate Test. Animals were injected with 40 mg/kg of methylphenidate (Novartis, SP., Brazil) I.P in dissolution with sterile saline, immediately; they were positioned in a circular Open Field (diameter 90 cm x 35.5 cm) during 30 min. The number of rotational activity and the direction was recorded, and subsequently analyzed (Ferro et al., 2005). Only, the animals with five or more ipsilateral-injury rotational of 360 degrees per minute were included in this study.

2.5 Isolation and Characterization of ADhMSCs

The ADhMSCs used in this study were obtained from abdominal adipose tissue of a healthy female patient that submitted to the liposuction procedure. Patient accepted donate it to research, through a signed Patients Consentient Term at Santa Casa de Misericórdia de Porto Alegre Hospital (Research Ethics Committee approval n0882968). The tissue derived was washed three times with Phosphate-buffered Saline (PBS) using a separate funnel then, it was placed into a Falcon tube for enzymatic treatment with collagenase solution Type I (3 mg/mL, 250 U/mg) dissolved in Dulbecco’s Modified Eagle’s Medium (DMEM, low glucose) (Sigma, St. Louis, MO., USA). Subsequently, the cells were incubated in a water bath at 37°C for 30 min (vortexed each 10 min). The double volume of DMEM with 10% of fetal bovine serum (FBS) (Cultilab, SP., Brazil) was used to inactive the collagenase solution. Next, cells were centrifuged at 600 x g for 10 min and resuspended in an erythrocyte lysis solution (150 mM NH4Cl, 10 mM NaHCO3 and 1 mM ethylenediamine tetraacetic acid (EDTA)) diluted in ultrapure water 1:1 (v:v)
with simultaneous mechanical shaking and incubated for 10 min at room temperature. After new centrifugation (600 × g, 10 min), cells were resuspended in a known volume of DMEM 10%FBS and counted by trypan blue exclusion. 5.2 x 10^3 cells/cm^2 were seeded in six-well plates and cultured at 37 °C in a humidified incubator with 5% CO2 being covered by 3 mL of culture medium. The replacement of DMEM was developed 48 hours later the appearance of adherent cells and the medium was changed each 4 days. Cultures were passaged using 0.25% trypsin and 0.01%EDTA (Invitrogen, Waltham, MA., USA) when the cells reached semi-confluence.

2.6 In vitro Differentiation of ADMSCs

For adipogenic, chondrogenic and osteogenic differentiations, the cells were seeded at a density of 2.2 x 10^5 cells/cm^2. Differentiation medium (Gibco, Grand Island, N.Y., USA) was added and replaced each 4 days, at least, for four weeks. The differentiation medium was changed every 3 days. Completed this term, cells were washed with PBS and fixed in 4% of paraformaldehyde (PFA) for 30 min at 2-8ºC. Cells were stained with 1% Alcian Blue solution prepared in 0.1 N HCL for 30 minutes, 4% Oil Red O solution in Isopropyl alcohol for 5 minutes and 2% Alizarin Red S in water (Ph: 4.2) for 2-3 minutes for chondrogenic, adipogenic and osteogenic assays, respectively. The pictures generated by a BX-50 Olympus microscope with optical lens (10X/0.30 Ph1-UplanFl), interfaced with a camera Motican 2500 (Olympus, Hamburg, Germany) and compared with the control culture, that cells that received only the standard culture medium (DMEM 10% FBS). Also, the phenotype of ADhMSCs was examined by flow cytometry equipped with 488 nm argon laser (Becton-Dickinson, San Diego, CA, USA) using a CellsQuest software. Specific antibodies for human's proteins CD14, CD34, CD44, CD45 and CD105 (Invitrogen, Waltham, MA., USA) were employed. At least 10.000 events were collected.

2.7 Intra-Striatal ADhMSCs Transplantation.

The protocols used in this study were approved by the Ethics Committee on Animal Use (CEUA) of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), under the number 176-15, following the resolutions of the CONCEA. The NIH “Guide for the
Care and Use of Laboratory Animals” (NIH publication N 80–23, revised 1996) was followed in all experiments. For transplantation, the ADhMSCs were used between passages 4 and 7. To collect the cells, the culture flasks were washed twice with PBS and trypsinized to remove the cell-plastic adherence. After, an enzyme inactivation with a double volume of DMEM 10% FBS was employed. Cells were centrifuged at 600 x g and the pellet was resuspended in PBS to procedure a counting by a Neubauer chamber. Finally, a known number of cells were centrifuged more on time and resuspended in PBS. The samples were maintained at low temperature (2-8ºC) at least two hours before transplantation.

Stereotaxic (Kopf Instruments, C.A., USA) surgeries of hMSCs grafting were conducted one day after Methylphenidate Test. Under deep anesthesia using a mixed solution containing 90mg/Kg of Ketamine and 10 mg/Kg of Xylazine, animals were injected I.P. Stereotactic coordinates were used to identify the striatum in the left hemisphere (AP: 0.5 mm, ML: +3.2 mm and DV: -4.5 mm). A microsyringe of 10 µL (701 N, Hamilton, Sigma, St. Louis, M.O., USA) was employed to administrate an infusion of 2 x 10^5 cells in 6 µL of PBS with a rate of 1.0 µL/min, totaling 6 min (Schwerk et al., 2015). The Hamilton syringe was left in place for 5 min after grafting to prevent leakage after being withdrawn. The control group received the same volume and equal rate of administration of the vehicle infusion (sterile saline). The incision was disinfected with iodopovidone and animals were supervised until recovery, controlling their weight and monitoring the pain sensation. Additionally, right after surgery, an injection I.P of Tramadol was administrated with doses of 12.5 mg/Kg.

2.8 Progressive Aerobic Treadmill Training

The animals exposed to the exercise training were previously acclimated for 3 days in the treadmill (Projetos AVS, SP., Brazil) with a speed of 9m/min for ten minutes, before any surgical procedures. They were prescribed with a dynamic progressive aerobic exercise with moderate intensity and a speed of 16 m/min four days after hMSCs grafting surgical process. The physical regimen consisted of 30 minutes initially, once by day on the treadmill for five sessions per week. Was added 10 minutes per week up to complete 60 minutes per session, then was maintained this intensity for two weeks. To ensure the exercise training, rats received an electrical shock of
0.5 mA, if they stopped at the base of the equipment. The total period of training was four weeks. The speed and the percentage of incline were not modified throughout the study period (Landers et al., 2014; Nunes et al., 2013).

2.9 Foot-Fault Walking Task

The Footfault task is a functional test that assesses the motor impairments of limbs during locomotor activity, including the motor coordination performance and the accuracy of paw placement (Schaar et al., 2010; Silvestrin et al., 2009). The animals were placed in the center of an elevated metal square opening grid with a measure of (4cm²); the dimension of the equipment was (50cm x 50cm x 50cm). They were free to explore during five minutes, as soon as this time was completed the animal was removed to its specific cage. This test was developed in a room with light and sound reduced. After each rat, 70% ethanol was used to clean the grid.

The slips of each limb and the number of ipsilateral-injury spins were registered by a micro-camera Microsoft® Life Cam VX-800 positioned between 40°-60° degrees below of the equipment. A foot-fault was considered if the paw dropped into the grid or if the paw was properly positioned but during the weight support phase, it slipped into of one orifice of the grid (Baskin et al., 2003; Starkey et al., 2005; Zhang et al., 2002). The videos were recorded and analyzed afterward frame by frame with the aid of an opened video analysis program, Tracker Video Analysis and Modeling Tool (Tracker, version 4.92). The total slips of each limb were obtained and then compared between the groups.

2.10 Histological Analysis

Sixty-three days following the 6-OHDA lesion surgery and upon completion of the motor behavioral assessment, the sacrifice of rats was developed through perfusion procedure. Rats were anesthetized with a solution of xylazine (10 mg/kg) and ketamine (100 mg/kg) injected I.P. Then, were transcardially perfused with 1mL of heparin injected into the left ventricle, followed by freshly prepared 4% PFA (at room temperature) in 0.1 M sodium phosphate buffer (pH 7.4) with a total volume of 350 mL using a peristaltic pump (Control Company,SP., Brazil)(Tao-Cheng et al., 2007; Zancan et al., 2017). Carefully, the brain was removed and placed in PFA for overnight (4°C). Subsequently, brains were transferred to a solution of sucrose 30% during 72 hours for cryoprotection. All samples were cooled with liquid nitrogen and stored at -85°C.
Coronal slides sectioning of the tissue was carried out on a CM3050S Cryostat from Leica Microsystems (Buffalo Grove, IL; USA) at -23°C, with a thickness of 50µm, samples obtained were preserved in a solution of PBS with sucrose at 30% in microtiter plates and stored at -20°C for the previous immunostaining.

*Tyrosine hydroxylase immunofluorescence*

The free-floating technique was used to stained coronal sections through by immunofluorescence for tyrosine hydroxylase (TH); the procedure was conducted in 24-well culture plates. Tissue sections were initially washed 5x5 min with PBS and then pre-blocked with 1% bovine serum albumin (BSA) and 0.025 of Triton X-100 in 0.1 M PBS for 30 min to enhance permeabilization.

Coronal slides were incubated with a polyclonal primary antibody, mouse anti-tyrosine hydroxylase (1:500; Invitrogen, cat# P21962) with 1% BSA and 0.025 Triton X-100 in 0.1 M PBS at 4°C for 24 hours in a microplate shaker. On the following day, slides were washed in PBS four times for at least 10 min each time and incubated with a secondary antibody (1:400 donkey anti-mouse IgG (Invitrogen, A-21202) Alexa Fluor® 568) and 0.025 Triton X-100 in 0.1 M PBS for an hour and a half in the microplate shaker. All the incubations were done at room temperature unless referred otherwise. In the final step, sections were mounted with Vectashield® (Vector Laboratories, cat# H-1000) mounting medium on glass slides with an overslipped. At least five sections of SN were analysis for TH immunofluorescence for two rats per group. Fluorescence was detected in an Olympus IX51 U-RFLT Inverted Microscope (Olympus Corporation, USA). The 510-550 wavelength laser was used for exciting the TH samples. The Olympus DP controller 3.3.1292 software was used for photographic capture. Two set of photos were taken at 4 x and 20x objectives. All technical parameters were maintained for shooting the different samples.

2.11 Statistical Analyses

All statistics were analyzed using IBM®SPSS® Statistics ver 20.0. All variables were expressed as “mean (SD)”. All data were initially subjected to Shapiro–Wilk test to determine the normal distribution of data. Subsequently, the comparison among the
groups were evaluated statistically using one-way analysis of variance (ANOVA) test. Statistical significance was preset at p< 0.05.

3. Results

3.1 ADMSCs Morphology

The micrograph in Figure 2 showed the morphology of the ADhMSCs during the isolation and culture procedure and fibroblast-like morphology (Fig.2a).

3.2 Feasibility of use and Differentiation Potential of ADMSCs- In vitro.

In addition, was demonstrated after 3 weeks a satisfactory adipogenic differentiation by the presence of lipid vacuoles (Fig.2b). The round constitution and the formation of extracellular matrix corroborated the presence of chondrogenic cells (Fig.2c) and finally, the osteogenic differentiation was determinate owing the appearance of calcium phosphate precipitates (Fig.2d) through Oil Red O staining, Alcian blue stain, and Alizarin Red S staining, respectively. These tests demonstrated the pluripotency capacity of the cells used in the present study. In this sense, to demonstrated the feasibility of our cells was developed a Flow The results of it confirmed the nature of our cells through the lack of expression of CD45, CD14, and CD34 (Fig.3a-c) and positive staining for CD44, and CD105 markers (Fig.3 d-e)
3.3. Rotational Behavioral Analysis in the Methylphenidate Test
The behavioral response seen in the unilaterally 6-OHDA-lesioned rats was the ipsilateral rotational behavior toward the side of the lesion (left side) with methylphenidate in the Open-field. None of the sham animals presented rotations. In our experiment, the analysis of variance showed not significant differences in the quantity of rotational activity between groups, being apparent the same degree of severity in the dopaminergic lesion between each sample \([F(3,28)=0.37, p=0.77]\).
3.4 Reduction in Rotational Activity was Mitigated after the Exposure to Treatments

Between all groups the number of ipsilateral turns were significantly different by one-way ANOVA [F (3,28)=11.7, p=0.00)]. Tukey HSD post hoc test revealed that the non-treated group had a higher number of rotations in contrast with the treatments categories. It was appreciable then, the decrease of this pattern of behavior in all treatment groups, 6-OHDA+exercise (3.63(1.59) p=0.00) 6-OHDA+Cells (4.88 (1.45) p=0.002) and 6-OHDA+combined (3.38 (2.20) p=0.00) during the performance of the Foot-fault task. There was not a significant difference among treatments groups regarding this variable (Fig.4b). No contralateral rotation was seen in any group.
3.5 Both Therapeutic Strategies improved Locomotor Performance.

A significant difference in the number of foot slips was found in the comparison among all experimental groups [F(4, 36)=22.3, p=0.00]). In the Tukey HSD post hoc test, the injured group had a significantly higher proportion of slips during the task than those allocated to sham or experimental treatments (p<0.001) (Fig. 5a). In the comparison of the treatments versus the sham group, just the 6-OHDA+cells group was statistically different (p=0.04) (Fig. 5a). We did not find significant difference between 6-OHDA+exercise and 6-OHDA+combined groups when compared among themselves (p=0.17) or with sham category (p=0.98, p=0.38, respectively (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Injury</td>
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<td>4.91</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
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<td>1.59</td>
<td>&lt;0.001</td>
<td>8</td>
</tr>
<tr>
<td>6-OHDA+Cells</td>
<td>4.88</td>
<td>1.45</td>
<td>0.002</td>
<td>8</td>
</tr>
<tr>
<td>6-OHDA+Combined</td>
<td>3.38</td>
<td>2.20</td>
<td>&lt;0.001</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>5.69</td>
<td>4.14</td>
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<td>41</td>
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</tbody>
</table>

**Table 1** - Rotational Pattern in the Foot-fault Task. Total turns were compared among the groups; p-values row represent the Tukey comparison between the injured group and the sham or each of the therapy groups. p<0.05.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
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<td>6.4</td>
<td>&lt;0.001</td>
<td>9</td>
</tr>
<tr>
<td>Injury</td>
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<td>15.3</td>
<td>-</td>
<td>8</td>
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<tr>
<td>6-OHDA+Exercise</td>
<td>28.5</td>
<td>8</td>
<td>&lt;0.001</td>
<td>8</td>
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<tr>
<td>6-OHDA+Cells</td>
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<td>12.37</td>
<td>&lt;0.001</td>
<td>8</td>
</tr>
<tr>
<td>6-OHDA+Combined</td>
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<td>13.1</td>
<td>&lt;0.001</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>44.78</td>
<td>20.25</td>
<td></td>
<td>41</td>
</tr>
</tbody>
</table>

**Table 2** - Total Number of Foot-slips. Foot slips were compared among groups; p-values in each row represent the Tukey comparison between the non-treated group regard to all experimental categories. p<0.05.
3.6 Therapeutic Strategies Tested Demonstrated an Improved the Exploratory Behavior

Significant differences were found in the number of foot-slips among the forelimbs and hindlimbs in the univariate analysis of variances in all groups (p<0.001), being greater the faults-mistakes in the first ones, this result supports findings reported in other studies about the relevance of forelimbs in the exploring capacity of rats. Regarding this variable, only the 6-OHDA+ exercise and the combined groups obtained fewer errors. Despite the cerebral unilateral lesion, there were no differences in the number of foot faults between corporal hemispheres in none of the groups (p=0.06) (Fig.5b-c).

**Fig.4.** Rotational Behavior. (A) The graph indicates the mean of the number of rotational pattern among the groups in the Open-Field (before therapeutic intervention). There was no significant difference in the degree of severity among each other. The sham sample does not register rotations. (B) The number of rotations were decreased in all the therapeutic categories during the development of the Foot fault task (p<0.001) by 1-way analysis of variance, Tukey’s multiple comparisons test (after exposure to treatments).
Fig. 5. The motor performance was improved in all treatment groups. (A) Rodents submitted to therapeutic interventions exhibited a decrease in the number of faults slips in contrast with non-treated sample p<0.001. A statistical difference only was found among the comparison of 6-OHDA+cells group versus sham category p=0.045. No significant difference was found in the correlation between the others treatment categories p=0.17. Behavioral testing was analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey HSD post hoc test post. Regard to Exploratory Behavior. (B) The animals in all samples exhibited significant differences in the forelimbs and hindlimbs foot-faults in the analysis of variance p<0.05. (C) Rodents did not show significant differences between slips among right and left paws foot slips p>0.05.
3.7 Staining Analysis demonstrated Dopaminergic Depletion in the Injured Side

In the present study, we observed a decrease in the levels of TH expression in the lesioned hemisphere in contrast with the healthy one in the injured groups by immunofluorescence analyses. It was visually identified a probable preservation of dopaminergic neurons in both SNc and the fibers that constitute and underlie substantia nigra pars reticulata in the treatment groups respect to rodents non-treat (Fig.6).
Fig. 6. Site-specific decrease in Tyrosine Hydroxylase staining. Immunofluorescence for tyrosine hydroxylase (red) in the substantia nigra compacta and substantia nigra reticularis. It is visually apparent the reduction on the injured sample in comparison to the others groups. Scale for smaller pictures = 200 μm.
4 Discussion

PD is a complex neurodegenerative disorder characterized by motor and cognitive symptoms, owing to the neuronal loss in the brain. Many elements, including aging, genetic susceptibility, lifestyle and environmental factors play a relevant role in the onset of this pathogenic process. Recent advances in research have contributed to understanding the mechanisms that are enrolled in the nigral dopaminergic cell death, framed by oxidative stress, mitochondrial dysfunction, genic mutations, and neuroinflammation process (Conte et al., 2013; Magrinelli et al., 2016). At present, only symptomatic treatments had been developed, being not enough to halt the disease progression. Thus, in the present study, we explore the retrieval degree in the motor function of PD model rats induced by 6-OHDA, using the combination of both therapeutic approaches.

In our work, we demonstrated through the morphologic and biophysical analyses the authenticity of our cells based on the high capacity to adherence, the ability of multipotent differentiation (into osteoblasts, adipocytes, and chondrocytes) (Fig. 2), and the positive expression of CD44, and CD105 markers (Fig.3). These parameters established by International Society of Cellular Therapy were used to confirm the accuracy of our cells as MSCs (Dominici et al., 2006; Pittenger et al., 1999).

Regarding the neurobehavioral data, the methylphenidate challenge demonstrated the presence of injury in all experimental categories, except the sham group. This outcome was confirmed by the visual inspection of the immunofluorescence staining of TH-positive neurons, where a less reactivity was detected in the lesioned side in contrast with the unaffected one (Fig.6). Similarly, after the exposure to the therapeutical approaches, we found a significant decrease in the number of ipsilateral rotations toward the injury-side in all treatment groups, especially, in the 6-OHDA+exercise and 6-OHDA+combined samples respect to the non-treat rats, where was evident the persistence of motor impairments, particularly, in paws placing and motor coordination (Fig.4). This outcome confirms the effectiveness of these therapeutic approaches, either in combination or used individually, for treating the imbalance of dopaminergic levels, resulting in a decrease in rotational pattern. Based on previous studies about the use of human mesenchymal stem
cells (hMSCs) for treatment neurodegenerative conditions, it has been recognized the potential benefits of this type of cells in owing to the self-renewal, multipotency capacity, and the high cellular differentiation ability (Han et al., 2017). The functional improvements recognized in our rodents might have been supported by the capacity of these cells to synthesize and secrete both, neurotrophic and growth factors such as: the brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), basic fibroblast growth factor (bFGF), glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF). These components are essential to preserve the rate of survival neurons, improve the microenvironmental conditions and exert a protective function (Park et al., 2015; Zarrinpour et al., 2017). Despite we did not analyze the levels of these factors, studies using the therapeutic strategies here worked have revealed that are crucial to induce motor recovery and functional independence in rodents.(Bardoni et al., 2007). In this sense, it has been also showed that aerobic exercise training leads to a better control of GABAergic interneurons, decreasing thus, the motor incoordination and the clear deficits in the synchronism between dopaminergic pathways (Borrione et al., 2014). Additionally, studies in rodents exposed to aerobic training programs have identified the presence of Insulin-like growth factor 1 (IGF-1) and the peroxisome proliferator activated receptor-gamma coactivator protein-1alpha (PGC-1α). This neurotrophin and transcription factor, apparently, have a relevant role in the preservation of the functionality and the protection of neurons being able to boost the benefits of the cellular graft, aiding with the synaptogenesis and neurogenesis processes (Fisher et al., 2008; Petzinger et al., 2015).

Certainly, one of the mechanisms that are implicated in the recovery of motor symptoms in PD is the restoration of the DA levels. In this case, the reinstatement of nigral dopaminergic neurons through cellular proliferation, the promotion of DA release from those remnant neurons and the stimulation of neural dopaminergic precursor cells are vital elements that ameliorate this locomotor symptomatic profile (Berg et al., 2015). In this context, it is possible that both therapeutic approaches caused positive regulation in DA levels and protective functions against dopaminergic depletion leading to an improvement in motor performance, however, deeper analyses should be performed to corroborate this assumption, due to was not possible tested it in our study.
In addition to the release of neurotrophins and the stimulation of the neuronal plasticity, the mitigation of aberrants corticostriatal glutamatergic and dopaminergic synapses have been reported from both therapeutic strategies, which can partially explain the observed improvement of motor and coordination deficits in our rats. (Ahmed et al., 2016; Pantcheva et al., 2015; Ye et al., 2007). These morphology-structural adaptations were already demonstrated by Wang et al. they concluded that long-term aerobic programs in Parkinsonian rats improve functional connectivity and reintegration between motor cortex with basal ganglia and cerebellum (Wang et al., 2015). Another effect from treadmill exercise was detected in Parkinsonian mice induced by 1,2,3,6-tetrahydropyridine (MPTP) where was showed an augment in D2 receptor and a decrease the rate of dendritic spine loss (Toy et al., 2014; Vučković et al., 2010). Taken together all these reports support our findings that exercise and cells transplantation are apparently two promising approaches in the treatment of motor function in PD. Nonetheless, in the present work, we identified a better motor performance and exploratory behavior in the rodents underwent exercise training when compared to cells transplantation per se. This overview confirms the superior and broad benefits of the exercise as a tool in the reinstatement of motor functionality in PD (Fig.5).

Another element that has associated with the decrease in motor deficits in patients and animal models of PD is the modulation of neuroinflammatory response through the reduction in the release of pro-inflammatory cytokines and the decrease of oxidative stress (Spielman et al., 2016; Zhang et al., 2013). Moreover, the strengthening of the neuronal microenvironment is a key effect that is related to an increase of the rate survival of injured neurons. In this context, both approaches have been implicated in the structural support to the extracellular matrix, the release of blood-derived factors, increased oxygen saturation and the contributions in the discard of neurotoxic elements in the brain, being fundamental to facilitate the intrinsic restorative processes (Dooves et al., 2016; Ploughman, 2008).

Despite our initial assumption to find a higher motor recovery in the 6-OHDA+combined group as compared to the other isolated treatment categories; our data from behavioral testing showed no significant difference respect this statement (Fig.5). This outcome
might be attributed to the powerful effects of exercise already mentioned. In this line of thought, we consider that the exercise outgrew the effects of the cellular therapy, achieving a better result in the locomotor patterns. The unsuccessful differentiation process into dopaminergic neurons or glial cells could be another explanation for the absence of better motor results in this particular group, in this case, is possible that the transplanted hMSCs might not have undergone a differentiation towards functional cells, decreasing thus the effectiveness of this treatment category. Although is probable that the cells grafted might differentiate in glial cells, it is from this point of view is important to highlight that even though glial cells have an essential role in the neuro-environmental control and differentiation process, its action on the production and restitution of DA is not enough, being persistent the presence of motor symptoms (Mena and García de Yébenes, 2008; Vila et al., 2001). This notion also could give an explanation about the absence of a better performance of the combined group during the development of the functional test here used. Additionally, we consider that quantity of cells that we grafted could be a determinant factor in the level of recovery in those groups that received certainly the cells. Some authors have been reported a linear relationship between the number of cells grafted and the degree of the improvement in the grade of functionality in animal models of PD (Haobam et al., n.d.). From this nexus is possible to infer that a major motor functionality level could have been obtained if more cellular resources would have been implanted.

Unfortunately, to our knowledge, there is no evidence that compares the efficacy of these therapeutic strategies in conjunction or separately. Being this the main gap in current knowledge for which this study was developed. From this experimental design, we failed to clarify the mechanisms by which our combined group was not superior on the motor performance respect to the other categories of treatment. Notwithstanding, we demonstrated that both therapeutic approaches have a positive effect on locomotor recovery and dopaminergic depletion.
5 Conclusion

The current study provided experimental evidence that supports the therapeutic benefit of ADMSCs and most especially the PE for the mitigation of motor deficiencies and the improvement in locomotor performance in an animal model of PD. These outcomes here represented are fundamental evidence in favor of their potential effectiveness as disease-modifying treatments that from of multiples neuro-plasticity mechanisms enhanced functionality in our rodents. Despite that are widely reported the activation of endogenous pathways that support the relieve of motor symptoms through the therapeutic strategies here worked, these are not well understood and elucidated what encourage the development of more studies in this area, allowing a better comprehension of the interrelation of exercise as an adjuvant in the cellular transplantation.

In the present work, we did not find better results in motor performance in our combined group in contrast with the isolated therapies. This result, against our initial assumption, could be associated with the dynamic and particular properties of each therapy by its own, a relationship that deserves to be more deeply studied, identifying thus possible mediating ceiling effects by these two therapeutic approaches.

Nonetheless, we consider of utmost importance the development of other studies that inquiry in a deeper way the neuronal adaptations induced by these treatment strategies. In the same way, it would be interesting to assess the potential of other modalities of exercise to heighten the neuroplastic and neuroprotective effects of therapies based on cellular transplantation in different neurodegenerative conditions as PD.

6. Acknowledgments

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7. Funding

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8. Conflicts of interest: No potential conflicts of interest.

9. Highlights

- The hydroxydopamine (6-OHDA) induce motor disturbances in exploratory behavior.
- The Foot-Fault Task is a sensible test to determine dopaminergic lesion in rodents models of PD.
- The combination of exercise and mesenchymal transplant did not generate synergic effects.
- The aerobic exercise and cellular transplantation improve locomotor performance.

10. References


Human adipose-Derived Mesenchymal Stem Cells Improve Motor Functions and are Neuroprotective in the 6-Hydroxydopamine-Rat Model for Parkinson’s Disease when Cultured in Monolayer Cultures but Suppress Hippocampal Neurogenesis and Hippocampal Memory Function when Cultured in Spheroids. Stem Cell Rev. Reports 11, 133–149. doi:10.1007/s12015-014-9551-y


CONCLUSION

The literature review and empirical study that were part of the present work support the approach about the promising and powerful effects of hMSCs transplantation and PE in the treatment of motor symptoms in the PD. Several studies cited in the present work, suggests a wide variety of mechanisms including the release of different neurotrophic factors, the stimulation of regenerative processes, the restitution of neurotransmitters, reinstalling of damaged brain areas and the constitution of pathways that aid to remove toxic components derived from the neuropathophysiological metabolism, ensuring the improvement in locomotor functionality. In this sense, these mechanisms of action boosted by the therapeutical strategies here used promotes the increases in DA levels, the boost of neurogenesis, the dopaminergic circuits repair, the modulation of neuroinflammatory processes and the constitution of appropriate conditions to increase not only the function but also the rate survival of remaining dopaminergic neurons. This engram of beneficial effects that take part in the recovery and neuroplasticity process could support the results that we obtained in our experimental study and could be associated with the decrease in the rotational behavior induced by the Foot-Fault Task, the enhancement of motor coordination and paws placing in our rats.

Despite that our initial conception was not proved and we did not find synergic results in the combined group in contrast with the isolated therapeutical approaches, the outcomes here registered are evidence that reinforces the effectiveness of SCs transplantation and, especially, the exercise as a favorable strategies that are essential to overcome the locomotor deficits and improve the motor coordination recovery in rodents with dopaminergic lesion.

The results here exposed give rise issues that unfortunately we could not respond in this study and our outcomes are opposite respect to other ones that have revealed superior benefits in the conjunction of the approaches here worked in other neurological conditions. Is for that reason that we strongly recommend more studies that emphasize in the understanding of the reciprocal interrelationship between cells transplantation and the use of exercise as an adjuvant method, determining not only the effects on the locomotor performance but also in the cognitive processing. In addition, is relevant a
better insight of the factors that could influence in the efficacy of the combination of these methods, including those own to the cell culture and graft procedures and those belonging to the modality and the parameters of the exercise. Allowing the development of better and effective therapeutic strategies that work against to the progressive and the functional motor decline in PD.
APPENDIXES

1. ETHICS AND RESEARCH COMMITTEE´S APPROVAL

CEUA –COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANTIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 176/15

2) DATA DO PARECER: 11/12/15

3) TÍTULO DO PROJETO:

Transplantes neurais e exercício físico aeróbio: combinação terapêutica em um modelo animal de doença de Parkinson

4) PESQUISADOR RESPONSÁVEL:

Alcyr de Oliveira

5) RESUMO DO PROJETO:

Trata-se de um estudo que tem por objetivo avaliar os efeitos da terapia combinada de transplantes neurais de células tronco e exercício físico aeróbico sobre o comportamento motor, memória, neurogênese e a secreção de BDNF. Para tal, ratos Wistar adultos serão submetidos a um modelo de doença de Parkinson (DP) por meio da injeção unilateral de 6-OHDA e terão implantes de células tronco mesenquimais humanas acompanhado de um regime de exercício aeróbico. Os desfechos avaliados serão memória de reconhecimento (por meio do teste de reconhecimento de objetos), condicionamento aversivo contextual (por meio da esquiva passiva) e atividade motora (por meio do footfault test). Após a realização dos testes comportamentais, os animais serão eutanasiados e os cortes de encéfalo serão submetidos à análise imunoistológica para verificar o estado da lesão, proliferação de células implantadas e expressão de BDNF.
6) **OBJETIVOS DO PROJETO:**

Investigar os efeitos da adição de exercício físico aeróbico ao tratamento com transplantes de células-tronco mesenquimais humanas derivadas do tecido adiposo sobre a memória e performance motora no modelo animal de DP

7) **FINALIDADE DO PROJETO:**

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<td>Materiais e Métodos</td>
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<td>Cronograma para execução da pesquisa</td>
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<td>Referências Bibliográficas</td>
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8) **ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:**

9) **O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:**

<table>
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10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse:    B  C  D  X  E  

*Justifique:* Procedimentos que podem causar dor ou estresse exigindo o emprego de anestésicos, analgésicos ou ansiolíticos.

**Espécie:** Ratos Wistar machos  **Número Amostral:** 66

**Redução Amostral:**

- [ ] Sim  
- [x] Não

*Justifique:* 

**Substituição de Metodologia:**

- [ ] Sim  
- [x] Não

*Se achar necessário, justifique e sugira uma nova metodologia:*

**Aprimoramento da Metodologia:**

- [ ] Sim  
- [x] Não

*Se achar necessário, justifique e sugira aprimoramentos da metodologia:*

**Acomodação e manutenção dos animais:**

- [x] Adequada  
- [ ] Inadequada

*Se achar inadequada cite abaixo as melhorias necessárias:*

**Manipulação dos animais:**

- [x] Adequada  
- [ ] Inadequada

*Se achar inadequada cite abaixo as melhorias necessárias:*

**Analgesia dos animais (se aplicável):**

- [x] Adequada  
- [ ] Inadequada

*Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:*

**Anestesia dos animais (se aplicável):**

- [x] Adequada  
- [ ] Inadequada

*Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:*
Eutanásia dos animais (se aplicável):  

〣 Adequada  □ Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Local de Realização (Biotério/Labotarório): Laboratório de Fisiologia e Laboratório de Fisiologia Comportamental e Metabólica

Outra instituição: Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

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12) RECOMENDAÇÃO:

〣 Aprovado

□ Com Pendência

□ Não aprovado

Data de início: 02/16   Data de Término: 05/17

Comentários gerais sobre o projeto:

Projeto bem escrito, em tema relevante, bem justificado quanto a necessidade de uso de animais.
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All tables (including titles, description, footnotes)

Ensure all figure and table citations in the text match the files provided

Indicate clearly if color should be used for any figures in print Graphical Abstracts /Highlights files (where applicable) Supplemental files (where applicable).

Further considerations

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