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Human adipose-derived mesenchymal stromal cells increase endogenous neurogenesis in the rat subventricular zone acutely after 6-hydroxydopamine lesioning

ANNE SCHWERK[1], JENNIFER ALTSCHÜLER[1], MANFRED ROCH[2], MANFRED GOSSEN[3], CHRISTINE WINTER[4], JÜRGEN BERG[1], ANDREAS KURTZ[2,5] & BARBARA STEINER[1]

[1]*Department of Neurology, Charité University Medicine, Berlin, Germany,* [2]*Berlin-Brandenburg Centre for Regenerative Therapies, Charité University Medicine, Berlin, Germany,* [3]*Institute of Biomaterial Science and Berlin-Brandenburg Centre for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Teltow, Germany,* [4]*Department of Psychiatry, Technical University Dresden, Dresden, Germany, and* [5]*Seoul National University, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul, Republic of Korea*

Abstract

Background aims. In Parkinson's disease (PD), neurogenesis in the subventricular zone (SVZ)–olfactory bulb (OB) axis is affected as the result of the lack of dopaminergic innervations reaching the SVZ. This aberrant network has been related to the hyposmia of PD patients, which is an early diagnostic marker of the disease. Consequently, much interest arose in finding mechanisms to modulate the SVZ-OB axis. Direct modulation of this axis could be achieved by transplantation of mesenchymal stromal cells (MSC), as it has been shown in rat and mouse PD models. However, the neurogenic effect of MSC in PD was thus far only analyzed weeks after transplantation, and little is known about effects immediately after transplantation. *Methods.* We assessed the acute neuroprotective and neurogenic effects of adipose-derived MSC transplanted into the rat substantia nigra in the 6-hydroxydopamine model of PD. *Results.* Three days after transplantation, subventricular neurogenesis was significantly increased in MSC-transplanted versus non-transplanted animals. Most MSC were found in the region of the substantia nigra and the surrounding arachnoid mater, expressing S100β and brain-derived neurotrophic factor, whereas some MSC showed an endothelial phenotype and localized around blood vessels. *Conclusions.* The acute neurogenic effects and neurotrophic factor expression of MSC could help to restore the SVZ-OB axis in PD.

Key Words: *adult stem cells, mesenchymal stromal cells, neurogenesis, Parkinson's disease, plasticity, regeneration*

Introduction

The incidence of Parkinson's disease (PD) is continuously increasing, with 1% of persons older than 60 years being affected, making it the most common neurodegenerative movement disorder [1]. Dopaminergic neurons in the substantia nigra (SN) undergo continuous degeneration in PD, and α-synuclein-positive inclusions in cell bodies and neurites (Lewy bodies) can be found in nigral and olfactory bulb (OB) dopaminergic neurons [2,3]. These Lewy body–like inclusions go together with a distorted neurogenesis in the subventricular zone (SVZ)-OB axis [4,5] and have been linked to hyposmia, which affects approximately 90% of all PD patients [6]. This implicates an abnormal SVZ-OB axis as an early sign of PD and an interesting therapeutic target. In recent years, regenerative strategies aimed to replace damaged dopaminergic neurons by transplanting fetal dopaminergic grafts or embryonic stem cells, mostly with non-ideal results. Neural progenitor grafts from embryonic stem cells failed to develop a dopaminergic phenotype [7,8], and postmortem studies revealed that some transplanted fetal nigral cells would also show inclusion bodies seemingly identical to Lewy bodies, implicating a spread of the disease even on allografts [9–14]. The transplantation of embryonic or induced pluripotent stem cells is additionally often complicated by the risk of teratoma development and graft-versus-host reactions [8,15,16]. Adult mesenchymal stromal cells (MSC) are a promising alternative, given their natural secretion of trophic

Correspondence: **Barbara Steiner**, MD, Clinic for Neurology, Charité University Medicine, Charitéplatz 1, 10117 Berlin, Germany. E-mail: barbara.steiner@charite.de

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factors and cytokines as well as their immunosuppressive and low tumorigenic properties on transplantation into allogenic and autologous hosts [17]. Because of their wide differentiation possibilities and undemanding expansion, MSC are applied in many disease models and numerous clinical trials [17,18], including PD [19]. MSC have also been shown to improve motor deficits and partially restore dopaminergic marker expression in the striatum and SN of parkinsonian rats [20–25]. These neurorescue effects of MSC can be partly ascribed to their influence on endogenous neuronal precursors. In PD, dopaminergic deafferentiation leads to severely impaired neurogenesis in the SVZ-OB axis [4,26], and dopaminergic cells in the OB also show Lewy body–like inclusions. Hence, finding mechanisms to directly modulate this axis could recruit endogenous repair mechanisms and hence greatly change the underlying pathological condition. MSC have the potential to affect this axis in PD models, by stimulating endogenous neurogenesis, as shown in the SVZ of 6-hydroxydopamine (6-OHDA)-lesioned rats after 23 days [21] and in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of PD [21,27]. Considering that neurogenesis tends to be regulated early [28], our study focused on the very early-term inductions in the SVZ.

Even though some studies show a transdifferentiation of MSC into dopaminergic neurons in PD animal models [23,29,30], it is commonly assumed that MSC exert their therapeutic effects through immunomodulatory and trophic factor release rather than through cell replacement [31]. Since the discovery of bone marrow–derived MSC, MSC have been isolated from almost all adult tissues, showing similar multidifferentiation and self-renewal abilities as bone marrow–derived MSC [32–34]. Adipose tissue, with its abundant vascularization, appears to be an especially rich source of MSC, containing up to 500 times more MSC than bone marrow [35,36] and shows similar immunomodulatory abilities [37,38]. The major advantages of adipose-derived MSC (AD-MSC) over bone marrow–derived MSC are their abundance and convenient isolation and cultivation. Furthermore, AD-MSC show higher proliferative capacity, later senescence and a higher level of neurotrophin secretion [25,39–43], making them the focus of current MSC research. It has been shown that AD-MSC can ameliorate PD symptoms by autologous transplantation into the rat SN 4 weeks after transplantation [44]. Thus far, the mechanisms of potential neuroprotective and regenerative effects of AD-MSC are not fully understood because they have only been investigated several weeks after transplantation, and neurogenesis induction has not been assessed for this cell type. Acute effects would be particularly interesting, given that MSC are often not detectable anymore at later time points, raising the question of how these cells actually achieve their results and where they disappear to.

Three-dimensional cell spheres show superior differentiation potential and trophic factor release compared with monolayer-cultured cells while mimicking a more natural cell microenvironment through cell and matrix interactions [45–47]. Adherent MSC—as opposed to MSC spheres (sMSC)—are anchor-dependent and consequently might have different adaptive potential in certain microenvironments. To explore the most effective AD-MSC transplant, we compared 2 MSC culture models (spheres versus adherent) that were based on *in vitro* and *in vivo* phenotype. Furthermore, because many studies indicated a gradual vanishing of MSC transplants [23,48–51], our aim was to find immediate effects that can explain the longer-term findings. Hence, this study investigated acute neurogenesis in relation to MSC phenotypic development in the 6-OHDA rat model.

Methods

Liposuction

Adipose tissue samples were obtained from a 21-year-old female patient during tumescent liposuction after informed written patient consent and according to the guidelines set by the Ethics Review Board of the Charité University Medicine Berlin. During tumescent liposuction, subcutaneous tissue was infused with saline containing anesthetic as well as epinephrine through a cannula; both liquid and tissue were then withdrawn by suction.

Herewith, we confirm that this study obtained ethics approval from the local ethics committee Landesamt für Gesundheit und Soziales, Berlin, and from the Ethics Review Board of the Charité University Medicine.

Isolation and cultivation of MSC

MSC were isolated from adipose tissue by rinsing the tissue samples extensively with 0.1 mol/L phosphate-buffered saline (PBS) containing no magnesium and no calcium ions (Biochrome AG, Berlin, Germany). The fat/PBS suspension was centrifuged at 350*g* for 5 min until 3 layers were obtained. After removing the upper oily layer and the lower aqueous phase, the fatty layer was extracted and digested for 60 min with 0.2 U/mL collagenase NB4 (Serva Electrophoresis, Heidelberg, Germany). Subsequently, the sample was centrifuged. The pellet was resuspended in 0.1 mol/L