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Neurotrophic factor-based strategies to enhance survival and differentiation of neural progenitor cells toward the dopaminergic phenotype

Stefano Di Santo, Hans R. Widmer

Abstract:

Parkinson's disease (PD) is a neurodegenerative disorder that presents with hallmark clinical symptoms of tremor at rest, bradykinesia, and muscle rigidity. Stem cell therapy has emerged as an experimental treatment for PD. However, optimizing the cell culture condition that allows enhanced survival and differentiation of cells toward the dopaminergic phenotype remains a logistical challenge. Here, we discuss the utility of a combination of neurotrophin-4/5 (NT-4/5) and glial cell line-derived neurotrophic factor (GDNF) in increasing the dopaminergic phenotypic expression of rat ventral mesencephalic (VM) tissue. Using organotypic explant cultures of fetal human ventral mesencephalon, we observed that NT-4/5 and GDNF as single factors, or in combination on DAergic neurons, increased survival and number of tyrosine hydroxylase immunoreactive neurons as well as the dopamine content in the culture medium. The application of specific neurotrophic factors, such as NT-4/5 and GDNF, as cell culture supplements or as adjunctive therapy to cell transplantation may achieve improved functional outcomes when contemplating cell-based regenerative medicine for PD.

Keywords:

Dopaminergic neurons, glial cell line-derived neurotrophic factor, neurotrophin-4/5, organotypic explant cultures, Parkinson's disease

Introduction

Parkinson's disease (PD) is characterized by loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta. Drugs therapies are currently available for the treatment of PD, however long-term pharmacological treatment is often accompanied by serious side effects. Stem cell therapy has been suggested as potent treatment for PD because they may represent as robust biological source of dopamine.

Stem Cell Therapy for Parkinson's Disease

It has been demonstrated that transplantation of human fetal nigral tissue is safe and may reinnervate the dopamine-depleted striatum in PD patients.[1-4] However, the survival of DAergic neurons limits the efficacy of this transplant strategy.[1] In this regard, nonfetal tissue sources of dopamine have been examined in an attempt to increase DAergic survival; along this line of investigation, induced pluripotent stem cells and embryonic stem cells have been evaluated as rich sources of DAergic neurons, but their potential to restore the striatum function is still under investigation.[5] Another interesting approach to increase the survival of DAergic neurons either in culture or following transplantation could be the use of neurotrophic factors. In this context, glial cell line-derived neurotrophic

Department of Neurosurgery, Neurocenter and Regenerative Neuroscience Cluster, Inselspital, University of Bern, CH-3010 Bern, Switzerland

Address for correspondence:
Dr. Hans R. Widmer, Department of Neurosurgery, Neurocenter and Regenerative Neuroscience Cluster, Inselspital, University of Bern, CH-3010, Bern, Switzerland.
E-mail: hanswi@insel.ch

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factor (GDNF) and neurotrophin-4/5 (NT-4/5) support the improved growth and survival of DAergic neurons.[6] Neurotrophic signaling pathways may be involved in these observed cell-surviving effects. In particular, GDNF is a member of the transforming growth factor-beta superfamily and promotes DAergic survival and differentiation by activating a multicomponent receptor complex called RET and the GDNF family receptor. Interestingly, GDNF increased high-affinity dopamine uptake in cultures of the fetal midbrain, improving DAergic viability and stimulating differentiation.[7,8] On the other hand, NT-4/5 belongs to the NT family and triggers a signaling pathway that involves the rat sarcoma-phosphatidylinositol 3-kinase-Protein Kinase B (Ras-PI3K-Akt) and the phospholipase C-gamma 1.[9,10] NT-4/5 can regulate the morphology and improve the survival of DAergic neurons in mesencephalic primary cultures.[11,12] The administration of GDNF and NT-4/5 increased the survival of rat ventral mesencephalic (VM) tyrosine hydroxylase immunoreactive (TH-ir) neurons along with stimulation of dopamine (DA) release in free-floating roller-tube (FFRT) cultures.[6] In addition, the ability of donor tissue storage in FFRT cultures supports the strategy to pretreat the cells with growth factors. Of note, in this respect, DAergic viability and functions have been restored in a rat model of PD by using fibroblast growth factor 2-mediated pregrafting expansion of primary VM precursor cells.[13,14] To date, most studies have only explored the effects of monotherapy of neurotrophic factors on DAergic cell survival. Here, we discuss our experiments evaluating the therapeutic potential of combined GDNF and NT-4/5 administration on VM tissue of human origin in an attempt to reveal the application of neurotrophic factors as cell culture supplement or as an adjunct therapy for cell transplantation in PD.

Neurotrophic Factor Treatment of Neural Progenitor Cells

We assessed the survival and differentiation potential of organotypic explants of the fetal human VM when cultured with or without GDNF and NT-4/5 singly or combined pretreatment. The combined pretreatment increased both cell number and DA content of TH-ir neurons better than using singular treatments of either neurotrophic factors alone. In addition, no difference is observed in culture volumes, while the level of lactate dehydrogenase in culture medium was decreased in all the treatment conditions. These findings advance our current knowledge on the contribution of neurotrophic factors for DAergic neurons in animal models.[6] Indeed, it has been demonstrated that the GDNF reduces apoptosis and stimulates DAergic fiber growth in DAergic neurons and fetal nigral grafts, respectively.[15,16] Moreover, GDNF and NT-4/5 reduce oxidative stress-induced cell death, which is implicated in PD and other neurodegenerative diseases, through an anti-apoptotic mechanism.[17,18] The reduction of apoptosis might be one of the events underlying the observed NT-4/5 and GDNF protective action in TH-ir neurons. In addition, the decrease of lactic acid dehydrogenase (LDH) levels after GDNF and NT-4/5 treatment offer an alternative mechanism. That LDH levels are not lower in the GDNF and NT-4/5 combined treatment than the single neurotrophic factor treatment suggests that the synergistic action of GDNF and NT-4/5 is not a direct action on cell death reduction, but rather an increase of maturation and/or differentiation of the TH-ir cells. Indeed, no significant variation in culture volume is observed between the different experimental conditions. In addition, the treatment with neurotrophic factors has no influence on the protein GFAP expression levels, as shown previously.[6] In contrast, this neurotrophic factor treatment could promote the survival and the growth of other neuronal cells including striatal and cortical GABAergic neurons,[9,19-21] suggesting the preferential effects of GDNF and NT-4/5 on the neuronal phenotype.

Several lines of investigation suggest that the pretreatment of DAergic neurons with neurotrophic factors may be a potential strategy for the PD treatment. In this context, we showed that both number of cultured DAergic neurons and DA levels increased after BDNF treatment.[22] In addition, the combined action of GDNF and BDNF promoted the survival of rat fetal nigral tissue.[16] There remain some discrepant reports on the effects of neurotrophic factors on cell survival and differentiation. For example, dopamine levels may correlate with the TH-ir cell number even though no growth factor treatment was applied; in contrast, a prominent increase in dopamine levels is achieved with the combined GDNF and BDNF treatment compared to the use of these neurotrophic factors individually.[16,23] Notably, a clinical study reported increased uptake of fluorodopa in 2 PD patients when graft is exposed to GDNF.[24] Further investigations are needed to better understand the benefit of GDNF and NT4/5 pretreatment in cultured cells of potential DAergic graft donors, as well as the effects of these neurotrophic factors as adjunct treatments in clinically relevant animal models of PD.

Conclusion

In conclusion, these findings on combined pretreatment with neurotrophic factors of DAergic neurons support the potential of this strategy for enhancing the survival and differentiation of neural progenitor cells as graft source for transplantation therapy in PD. Adjunctive use of neurotrophic factors with cell therapy may also reveal improved functional outcomes.

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Conflicts of interest

There are no conflicts of interest.

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