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Creatine supplementation improves neural progenitor cell survival in Huntington's disease

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Abstract:

Preclinical and clinical studies suggest that striatal transplantation of neural stem cells (NSCs) and neural progenitor cells (NPCs) may be an appealing and valuable system for treating Huntington's disease. Nevertheless, for a neural replacement to become an effective translational treatment for Huntington's disease, a certain number of difficulties must be addressed, including how to improve the integration of transplanted cell grafts with the host tissue, to elevate the survival rates of transplanted cells, and to ensure their directed differentiation into specific neuronal phenotypes. Research focusing on the translational applications of creatine (Cr) supplementation in NSC and NPC cell replacement therapies continues to offer promising results, pointing to Cr as a factor with the potential to improve cell graft survivability and encourage differentiation toward GABAergic phenotypes in models of striatal transplantation. Here, we evaluate research examining the outcomes of Cr supplementation and how the timing of supplementation regimes may affect their efficacy. The recent studies indicate that Cr's effects vary according to the developmental stage of the cells being treated, noting the dynamic differences in creatine kinase expression over the developmental stages of differentiating NPCs. This research continues to move Cr supplementation closer to the widespread clinical application and suggests such techniques warrant further examination.

Key words:

Creatine, creatine kinase, development, differentiation, GABA, Huntington's disease, neuroprotection

Introduction

Huntington's disease (HD) is a hereditary neurodegenerative disorder that presently affects over 20,000 people in the United States with an additional 70,000 living as carriers of the disease.[1] HD is known for its morbid pathology which involves the progressive degeneration of striatal neurons, specifically GABAergic interneurons, manifesting in fitful, involuntary movements coincident with deteriorating behavioral and cognitive functions.[2,3] Lethal and as-of-yet incurable, the disease results from a trinucleotide expansion in the gene *Huntingtin* which causes a buildup of mutant protein aggregates in the cytoplasm and nucleus.[4] However, the precise mechanism of its neurodegenerative effects remains elusive. Few effective treatments exist to stymie the progression of the disorder, with most available medications aimed at regulating the psychological and kinetic symptoms, making the need for new clinical treatments extremely vital.

The recent preclinical and clinical studies suggest that striatal transplantation of neural stem cells (NSCs) and neural progenitor cells (NPCs) may be an appealing and valuable system for treating HD.[5-16] In clinical trials, transplantation was shown to reduce hypometabolism in the striatum characteristic of the disease and to improve motor and cognitive functions up to 2 years after implantation, with evidence that improvements may endure as far as 5 years posttransplantation.[17,18] Nevertheless, for neural replacement to become an effective translational treatment for HD, a certain number of difficulties must be addressed, including how to improve the integration of transplanted cell grafts with the host tissue, to elevate the survival rates of transplanted cells, and to ensure their directed differentiation into specific neuronal phenotypes. Discovering the factors necessary to promote the survivability and differentiation of grafted cells is crucial to advancing transplantation therapies for HD and may also provide ancillary insights as to the utility of such factors in other, similar therapies.

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Creatine and the Bioenergetics of Neurodegeneration

The recent studies point to creatine (Cr) as a promising factor with the potential to improve cell graft survivability and encourage differentiation in models of striatal transplantation. Cr is a nitrogenous amine which can be reversibly phosphorylated by the creatine kinase (CK) family of isozymes to form phosphocreatine (PCr).[19,20] Cr and PCr function together as a cellular energy shuttle, transferring inorganic phosphate to ATP-starved regions under high metabolic demand. In the central nervous system (CNS), two common forms of CK are expressed: The brain-specific cytosolic form (BB-CK) and the ubiquitous mitochondrial isoform (uMT-CK).[21] The bulk of neural Cr reaches the brain by crossing the blood–brain barrier through the CRT transporter.[21] Prior investigations have revealed that neural degeneration in HD corresponds to a reduction in cellular energy metabolism in affected neurons, evidenced by a decrease in activity across mitochondrial complexes II and III.[22,23] Moreover, the quantity of neuronal PCr is reduced in Huntington's patients, suggesting that metabolic dysfunction may arise as a result of the disease's pathogenesis.[24] GABAergic neurons carry a high metabolic demand, and their proper function may depend on the maintenance of the Cr-PCr pathway. This information has informed recent efforts to improve regenerative therapies intended to replace affected GABAergic neurons.

Creatine Improves the Induction Rates and Robustness of Striatal Neural Progenitor Cells Based on Developmental Age

A recent study conducted by Andres *et al*. examined the efficacy of Cr supplementation as a translational method to improve striatal NPC transplantation. Noting the dynamic differences in CK expression over the developmental stage of differentiating NPCs, the study investigated whether Cr's effects also varied according to developmental stage.[25] E14 (early development) and E18 (late development) NPCs were extracted from rat embryos and cultured *in vitro* for 7 days with a chronic treatment of Cr (5 mM). A separate selection of E14 and E18 NPCs was treated with an acute, 24 h supplement of Cr (5 mM). Chronic treatment greatly elevated the density of GABAergic neurons in the Cr-treated NPC cultures as compared to controls, with the E14 culture experiencing a greater effect than the E18 culture. In the acute treatment, similar trends were observed in the induction of the GABAergic phenotype for E14 and E18 cultures. Cell numbers and total neuronal viability were not affected by Cr supplementation, but Cr did provide equal protection against metabolic insult for both groups, with an explicit protective effect on the survival of GABAergic neurons. Cr exposure also encouraged increased neuronal complexity in GABAergic neurons, elevating neurite length for both cultures and increasing the number of branching points for cells in the E18 culture but not E14 culture. This study establishes that Cr can be an effective factor in the promotion of GABAergic differentiation and a source of neuroprotection against metabolic insult. Of these two features, only the influence on induction rate is mediated by an NPC's developmental stage.

Considerations for Future Clinical Applications

Differentiation of NSCs and NPCs into metabolically active neuronal lineages necessitates developmental changes in their bioenergetic systems, and experimental evidence suggests that the CK phosphotransfer system may serve as a key element in this transition.[21,25] In striatal NPCs isolated from E14 (early) rat embryos, BB-CK and uMT-CK exhibited high rates of coexpression and colocalization in GABAergic cells after differentiation.[26] In addition, acute and chronic supplements of 5 mM Cr to culture media encouraged GABAergic differentiation without adversely impacting cell survival while simultaneously providing a neuroprotective effect.[25] Cr addition also appears to elevate cellular ATP levels significantly above those of non-Cr-exposed cells.[27] The clinical safety of Cr supplementation has been tested in three Phase II clinical trials carried out on symptomatic HD patients with results demonstrating this treatment bears no adverse effects.[28-30] Because Cr can cross the blood–brain barrier and has a low toxicity profile, Cr supplementation is an increasingly appealing paradigm for supporting cell-based therapies to treat HD.[31,32] The safety of Cr supplementation suggests that it could be implemented both as a pretreatment for NSCs and NPCs before transplantation and as a systemic aid to support graft survival, differentiation, and integration after the procedure.

It is necessary to consider that the pathological microenvironment in the striatum of HD patients may encourage the degeneration of grafted NPCs. Therefore, developing treatments that combat HD-related atrophy may also improve the success of graft survival. Bioenergetic dysfunction is a common marker of the pathogenesis of many neurodegenerative disorders and often involves compromised mitochondrial function, the release of reactive oxygen and nitrogen species, oxidative damage, Ca^{2+} accumulation, and cell death.[33,34] Oxidative stress can disable CK isozymes, resulting in a loss of BB-CK generated ATP-flux and a decrease in protection against the mitochondrial permeability transition through inactivation of uMT-CK.[35-37] Animal models of BB-CK and Cr deficiency display similar patterns of cerebral dysfunction to those observed in patients suffering from BB-CK or Cr-deficient disorders and, importantly, HD.[38-40] With decreased cellular CK activity and reduced mitochondrial function potentially encouraged by the microenvironment in which NPC transplants would be grafted, exogenous supplementation of Cr may improve graft survival by overcoming these conditions as Cr-supplemented cells display higher metabolic rates and cellular ATP concentrations than controls.[27] In addition, Cr may improve the integration of grafted cells with host tissues as Cr supplementation has been reported to encourage axonal growth in NH_4CL-treated rat neurons.[41,42]

The expression of CKs in the CNS varies over a cell's developmental age, and the most suitable donor age for transplantation of striatal NPCs has not been determined.[43-46] In the investigation conducted by Andres *et al*., NPCs isolated from E14 (early) and E18 (late stage) rat embryos were grown *in vitro* as dissociated cultures and treated with a regime of chronic Cr supplementation up to a final concentration of 5mM from DIV0-7 (day *in vitro*).[25] Cr addition resulted in an increased incidence of induction toward GAGA-ir neurons

for both E14 and E18 neurons as compared to controls, with GABAergic differentiation occurring at a significantly higher incidence in E14 cells. Moreover, the team's findings indicated that induction to the GABAergic phenotype was permanent as GABA-ir cell densities persisted for 3 days in culture without further Cr addition.[25] Functional GABA uptake was also increased in both E14 and E18 cultures, which corroborates previous data on the effects of chronic Cr addition in E14 cultures. Andres *et al.* also examined whether acute Cr supplementation for 24 h from DIV6-7 (day *in vitro*) would result in similar patterns of induction.[25] Although E18 cells displayed significant trends toward GABAergic induction in this acute treatment, E14 induction toward this phenotype was less pronounced. An MTT assay of both cell groups exposed to acute and chronic supplementation revealed that total neuronal cells and viability were not affected by Cr addition, which did not suggest that Cr provides general neuroprotective effects.[25] Importantly, the results of the study point to Cr as a factor that promotes the directed differentiation of NPCs toward the GABAergic phenotype.

The facility of Cr to stimulate the differentiation of NPCs to the GABAergic phenotype may have important implications for improving the efficacy of cell replacement therapies for HD. However, the timing at which this differentiation occurs can influence the fidelity of the resultant graft. Complete induction before transplantation reduces the subsequent survival of grafted cells and lowers the efficiency of host integration.[25] This temporal consideration suggests that it would be advantageous to supplement NPCs with Cr acutely before grafting while also treating the patient chronically before and after transplantation with exogenous Cr on account of the facility of Cr to cross the blood–brain barrier.

Cr supplementation also represents an appealing paradigm for transplantation strategies as it may provide neuroprotective effects in response to metabolic insult. When Andres *et al.* induced a metabolic insult in E14 and E18 cell cultures by depriving the cultures of serum and glucose, in cells that were supplemented with Cr, GABAergic cell loss was equally reduced across both E18 and E14 cells as compared to cultures that did not receive supplementation but did receive the insult.[25] Neuroprotective effects of Cr have been reported in experimental models of HD and other neurodegenerative diseases previously.[47-58] Importantly, Andres *et al.* demonstrate with these results that the developmental stage of the treated neural cells does not influence the present neuroprotective outcome of Cr supplementation.

In the investigation by Andres *et al.*, Cr exposure was also found to encourage morphological differentiation in treated NPC-derived neurons. Cr addition resulted in a significant increase in neurite length and the number of branching points for E18 neurons and a similar increase in neurite length, but not branching point, in E14 neurons.[25] As NPCs begin to differentiate into neurons, the spatial geometry of the cell body that houses the cellular phosphotransfer network becomes more intricate. Cr might indirectly facilitate morphological growth by increasing cellular energy reserves in the form of PCr and providing further substrate for energetic transactions of the phosphotransfer network to occur at a faster rate.[25] Andres *et al.* also tested whether morphological changes would be observed in cases of metabolic insult. No significant changes in neuronal complexity were found, suggesting acute metabolic stress outweighs the benefits to neuronal complexity provided by Cr supplementation.[25] However, the morphological effects that were observed under standard conditions indicate that Cr supplementation may help to improve graft integration with host tissues by promoting more complex morphologies in differentiating neural structures.

Conclusion

The developmental stage of NPCs does influence the success of Cr supplementation in promoting GABAergic differentiation but not its capacity to provide neuroprotective effects. The findings by Andres *et al.* point to Cr as an important factor in improving the efficacy of NPC or NSC-based regenerative therapies to treat HD and other neurodegenerative diseases. Future studies should determine if developmental stage mediates the effects of Cr supplementation in human NPCs and NSCs.

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Conflicts of interest

There are no conflicts of interest.

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