Growth Hormone Deteriorates the Functional Outcome in an Experimental Model of Huntington’s Disease Induced by 3-Nitropionic Acid

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Background and Purpose: Growth hormone (GH) has been frequently used to control the aging process in healthy individuals, probably due to its slowing effect on senescence-associated degeneration. Mitochondrial dysfunction is related to the aging process, and one of the chemical models of Huntington’s disease is that it can be induced by mitochondrial toxin. To investigate the potential application of GH to modify the progression of Huntington’s disease (HD), we examined whether GH can protect the functional deterioration by striatal damage induced by 3-nitropionic acid (3NP).

Methods: 3NP (63 mg/kg/day) was delivered to Lewis rats by osmotic pumps for five consecutive days, and the rats received intraperitoneal administration of GH or vehicle (saline) throughout the experiment. Neurological deficits and body weight were monitored. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was performed to further determine the mitochondrial activity in cultured N18TG2 neuroblastoma cells in vitro.

Results: 3NP-treated rats showed progressive neurologic deficits with striatal damage. Application of GH accelerated behavioral deterioration, particularly between day 3 and day 5, resulting in reduced survival outcome. The body weights of rats given 3NP were decreased, but GH did not affect such decrease compared to the non-treated control group. The effect of GH on cultured neuronal cells was a decrease in the MTT absorbance, suggesting a lower number of cells in a dose dependent pattern.

Conclusions: Those results suggest that application of GH to a 3NP-induced experimental model of HD deteriorates the progress of functional deficits, possibly disturbing mitochondrial activities.

Key Words: Growth hormone, 3-nitropionic acid, Huntington’s disease, Mitochondria.
signaling, mitochondrial function, and protein turnover, also noted with aging, overlap with the cellular pathways that are altered in HD.12

Growth hormone (GH) also begins to decrease by 14.4% every 10 years from the age of 20 years. At the age of 60 years, the level of GH is reduced to 50%, compared to that in the 20s.13,14 At the age of 65 years or older, 30% of people are deficient in GH. Thus, GH replacement therapy has been one of the practices to reduce the aging phenomenon.15 However, application of GH in neurodegeneration is rare.16 Therefore, in this study, we investigated whether GH can attenuate the striatal degeneration induced by 3NP. Since GH is known to have anti-aging effect, we hypothesized that application of GH can reduce neurodegeneration in a mitochondrial toxin-induced striatal degeneration model.

Methods

Animal model

Twelve Lewis rats (Japan SLC, Hamamatsu, Japan), weighing 300 to 320 g and aged 12 weeks, were used for the experiment. Animals were divided into two groups, a 3NP + vehicle (saline) group (n = 6) and a 3NP + GH group (n = 6). 3NP infusion was performed as previously described.11,17,18 In brief, rats were anesthetized with a mixture containing xylazine hydrochloride (Sigma, St. Louis, MO, USA, 4.5 mg/kg) and zoletil hydrochloride (Sigma, 90 mg/kg). An incision was made below the base of the neck, and an Alzet osmotic minipump containing 3NP was positioned under the skin. 3NP was dissolved in 0.1M phosphate-buffered saline (PBS, pH 7.4) and containing 3NP was positioned under the skin. 3NP was dis-
tween days 4 and 5. The mean value of neurological scores in the 3NP + GH group before death was 6 points, whereas rats in the 3NP + vehicle group showed survival until the final day of the experimental protocol and were given the highest score of 8 points (Figures 2 and 3).

GH failed to reduce the decline of body weight
The 3NP + GH group showed no further decrease in body weight compared to the 3NP + vehicle group. The initial mean body weight in the 3NP + vehicle group was 337.0 ± 16.8 g (mean ± standard deviation), whereas that of the 3NP + GH group was 340.7 ± 14.5 g, which was not different. At day 5, three rats with GH treatment died. The mean body weight in the 3NP + vehicle group decreased to 288.7 ± 16.7 g and 300.5 ± 4.9 g in survived rats with GH treatment. There was no significant difference.

Mitochondrial activity in cultured neuronal cells decreased by GH treatment
To further determine the deteriorating effect of GH on a 3NP-induced model, mouse neuronal cells were cultured and treated with GH. To determine whether there was an effect of GH on the cell viability or mitochondrial activity, an MTT assay was done, which can reflect both aspects in this experiment. From a concentration of 0.25 IU/mL or higher, the absorbance of the MTT assay decreased in a dose dependant manner (Figure 4). However, direct observation of GH treated cells with inverted microscopy did not show evidence of cytotoxicity, although the number of cells was decreased.

Discussion
In this study, we examined whether the growth hormone can prevent striatal lesions induced by 3NP in a model of neurodegeneration in HD. We could not observe a protective effect of GH when compared to the vehicle treatment control. Instead, the behavioral condition in the 3NP + GH group was more aggravated than that in the control group and half of the rats treated with GH progressed to death.

In cultured neuronal cells, GH did not proliferate but appeared to lead to differentiation. This finding suggests that GH did not seem to be cytotoxic to the cultured neurons. However, whether GH aggravates the mitochondrial dysfunction, suggested by decreased functional outcome, warrants further clarification.

GH controls the metabolism of proteins, carbohydrates, or
fatty acids, or22 increases lipolysis by PI3 kinase.23,24 Therefore, it can be expected that a redistribution of fat can alter the body weight and the monitoring of body weight also reflects the effect of GH. Assessment whether the change of body weight was related to the neurological deficit. However, our study did not show a correlation between the body weight and neurological score. It may be explained that the effect of GH on body-weight is independent or be interpreted that the experimental protocol may not be long enough to test such a long-term effect.

A decreased absorbance of MTT in cells treated with GH, possibly due to cell viability or mitochondrial dehydrogenase activity. GH binds to one of the proteins of the cytokine class I superfamily, which functions through tyrosine kinase and signal transducer and activator of transcription.25-27 However, it is unknown whether GH involves these signaling pathway). Yet, dose-dependent or cell proliferation/differentiation might be related to one of the possibilities. A low dose of GH increases cell proliferation, whereas a higher dose of GH inhibits cell growth but differentiation21,28,29 GH inhibits neural differentiation by down-regulating neurogenin-1 expression.30 A high dose of GH can increase PARP expression with increased cleavage fragment.31 Our experiment protocol does not support a protective effect of GH in 3NP-induced striatal degeneration; rather, dose-dependent or differentiating effect may alter the outcome of 3NP-treated rats.

Since 3NP causes mitochondrial dysfunction, it also affects energy metabolism, which is a common mechanism in neurodegenerative disorders.3 3NP was used to develop an HD model,1 and complex II mitochondrial dysfunction can comprise the specific changes found in the brains of HD.16,17,32 In HD, mutant huntingtin activates JNK in the hippocampus,33,34 and in the striatum through mechanisms similar to those observed in 3NP models.10,35 Accordingly, present study may provide indirect information regarding the use of GH for the therapeutic strategy in HD. Care needs to be taken with the application of GH in terms of dose.36-38 A smaller dose, instead of higher dose, was reported to be of benefit for the aging-related symptom complex.39

In this study, we attempted to test the protective effect of GH; however, the results showed opposite to the expectation, worsening by the GH in 3NP-induced mitochondrial toxicity. However, it cannot directly indicate that the difference in behavior is directly related to the difference in the striatal mito-
chondrial toxicity caused by GH. Considering that GH is in-
ternalized in the mitochondria that decrease respiratory chain activity. The present study may provide supportive evidence, at least, of the effect of GH on mitochondrial dysfunction.

Taken together with the previous reports and our experi-
ment, GH enhances the toxic effect of 3-nitropropionic acid in an animal model of Huntington’s disease. Those results warrant further careful consideration of GH and its clinical feasibility for the treatment of neurodegenerative diseases associated with mitochondrial dysfunction.

Acknowledgments

This work was supported by the Korea Health 21 R&D Project (A092058), and WCU Neurocytomics.

REFERENCES

1. A novel gene containing a trinucleotide repeat that is expanded and un-
stable on Huntington’s disease chromosomes. The Huntington’s Dis-
ease Collaborative Research Group. Cell 1993;72:971-983.
2. Bates G. Huntingtin aggregation and toxicity in Huntington’s disease. Lancet 2003;361:1642-1644.
3. Agrawal N, Patlou J, Slepko N, Apostol BL, Bodai L, Chang LW, et al. Identification of combinatorial drug regimens for treatment of Hun-
tington’s disease using Drosophila. Proc Natl Acad Sci U S A 2005; 102:3777-3781.
4. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, et al. Oxidative damage and metabolic dysfunction in Hunting-
ton’s disease: selective vulnerability of the basal ganglia. Ann Neurol 1997;41:646-653.
5. Tabrizi SJ, Cleeter MW, Xuereb J, Taamman JW, Cooper JM, Schapira AH. Biochemical abnormalities and excitotoxicity in Huntington’s dis-
ease brain. Ann Neurol 1999;45:25-32.
6. Beal MF. Mitochondria take center stage in aging and neurodegenera-
tion. Ann Neurol 2005;58:495-505.
7. Gu M, Gashi MT, Manu VM, Javoy-Agid F, Cooper JM, Schapira AH. Mitochondrial defect in Huntington’s disease caudate nucleus. Ann Neurol 1996;39:385-389.
8. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Stritt-
matter WJ, et al. Early mitochondrial calcium defects in Huntington’s disease are a direct effect of polyglutamins. Nat Neurosci 2002;5:731-736.
9. Brouillet E, Jacquard C, Bizat N, Brouillet E, Caboche J. Mitochondrial toxicity caused by GH. Considering that GH is in-
ternalized in the mitochondria that decrease respiratory chain activity. The present study may provide supportive evidence, at least, of the effect of GH on mitochondrial dysfunction.

10. Hands S, Snidanos C, Wytenbach A. Polyglutamine gene function and dysfunction in the ageing brain. Biochim Biophys Acta 2008; 1779:507-521.
11. Martin FC, Yoo AL, Sonksen PH. Growth hormone secretion in the elderly: ageing and the somatopause. Baillieres Clin Endocrinol Metab 1997;11:223-250.
12. Toogood AA, O’Neill PA, Shalet SM. Beyond the somatopause: growth hormone deficiency in adults over the age of 60 years. J Clin Endocrinol Metab 1996;81:460-465.
13. Harman SM, Blackman MR. Use of growth hormone for prevention or treatment of effects of aging. J Gerontol A Biol Sci Med Sci 2004; 59:652-658.
14. Cummings DE, Merriam GR. Age-related changes in growth hor-
monsecretion: should the somatopause be treated? Semin Reprod Endocrinol 1999;17:311-325.
15. Blum D, Gali D, Czudnochowski SN. Topological analysis of striatal lesions induced by 3-nitropropionic acid in the Lewis rat. Neu-
roreport 2001;12:1769-1772.
16. Bizet N, Hermel JM, Boyer F, Jacquard C, Créminon C, Ouary S, et al. Calpain is a major cell death effector in selective striatal degenera-
tion induced in vivo by 3-nitropropionatc implicatons for Hunt-
ington’s disease. J Neurosci 2003;23:5020-5030.
17. Mittoux V, Ouary S, Monville C, Lisovoski F, Poyot T, Conde F, et al. Corticostriatopallidal neuroprotection by adenosinemediated ciliary
neurotrophic factor gene transfer in a rat model of progressive striatal
degeneration. J Neurosci 2002;22:4478-4486.
18. Bantubungi K, Jacquard C, Greco A, Pintor A, Chiaro T, Tai K, et al. Minocycline in phenotypic models of Huntington’s disease. Neurobiol Dis 2005;18:206-217.
19. Lyuh E, Kim HJ, Lim J, Lee JK, Park KS, Yoo KY, et al. Dose-spe-
cific or dose-dependent effect of growth hormone treatment on the proliferation and differentiation of cultured neuronal cells. Growth Horm IGF Res 2007;17:315-322.
20. Moller N, Gjestedt J, Gornelsen, M. Fuglsang J, Djerhaus C. Effects of growth hormone on lipid metabolism in humans. Growth Horm IGF Res 2003;13 Suppl A:S18-S21.
21. Yamauchi T, Kaboragi Y, Ueki K, Tsuji Y, Stark GR, Kerr IM, et al. Growth hormone and prolactin stimulate tyrosine phosphorylation of insulin receptor substrate-1, -2, and -3, their association with p85 phosphatidylinositol 3-kinase (PI3-kinase), and concomitantly PI3-ki-
rase activation via Jak2 kinase. J Biol Chem 1998;273:15179-15176.
22. Vernon RG, Lindsay-Watt S. Possible role for PI3 kinase but not p70S6 kinase in regulation of lipogenesis by insulin and growth hormone in sheep adipose tissue. Biochern Soc Trans 1995;23:1908.
23. Helligren G, Albertsson-Wikland K, Billig H, Carlsson LM, Carlsson B. Growth hormone receptor interaction with Jak proteins differs be-
tween tissues. J Interferon Cytokine Res 2001;21:75-83.
24. Carter-Su C, Smit LS. Signaling via Jak tyrosine kinases: growth hormone receptor as a model system. Recent Prog Horm Res 1998;53:
61-82: discussion 82-83.
25. Han Y, Leaman DW, Watling D, Rogers NC, Groner B, Kerr IM, et al. Participation of Jak and STAT proteins in growth hormone-induced
signaling. J Biol Chem 1996;271:5947-5952.
26. Dubrovolsky G, Giacinti C, Pelosi L, Nicolotti C, Winn N, Barberi L, et al. Muscle expression of a local Igf-1 isoform protects motor neu-
eurons in an ALS mouse model. J Cell Biol 2005;168:193-199.
27. Scheppens A, Sirimanne ES, Breier BH, Clark RG, Gluckman PD, Williams CE. Growth hormone as a neuronal rescue factor during re-
covery from CNS injury. Neuroscience 2001;104:677-687.
28. Dolcet X, Soler RM, Gould TW, Egea J, Oppenheimer RW, Cornella JX. Cytokines promote motoneuron survival through the Janus ki-
rase-dependent activation of the phosphatidylinositol 3-kinase pathway. Mol Cell Neurosci 2005;22:2174-2184.
29. Winkler T, Sharma HS, Stalberg S, Badagian YD, Westman J, Ny-
berg F. Growth hormone attenuates alterations in spinal cord evoked potentials and cell injury following trauma to the rat spinal cord. An experimental study using topical application of rat growth hormone. Amino Acids 2005;19:363-371.
30. Ludolph AC, He F, Spencer PS, Hammerstad J, Sabir M. 3-Nitropro-
pionic acid-exogenous animal neurotrans and possible human striatal
toxin. Can J Neurol Sci 1991;18:492-498.
31. Liu Y, Dorow D, Marshall J. Activation of MLK2-mediated signal-
ing cascades by polyglutamine-expanded huntingtin. J Biol Chem 2000;275:19035-19040.
34. Apostol BL, Illés K, Pallós J, Bodai L, Wu J, Strand A, et al. Mutant huntingtin alters MAPK signaling pathways in PC12 and striatal cells: ERK1/2 protects against mutant huntingtin-associated toxicity. Hum Mol Genet 2006;15:273-285.

35. Garcia M, Charvin D, Caboche J. Expanded huntingtin activates the c-Jun terminal kinase/c-Jun pathway prior to aggregate formation in striatal neurons in culture. Neuroscience 2004;127:859-870.

36. Zamenhof S. Stimulation of the proliferation of neurons by the growth hormone I. Experiments on tadpoles. Growth 1941;5:123-139.

37. Hanci M, Kuday C, Oğuzoğlu SA. The effects of synthetic growth hormone on spinal cord injury. J Neurosurg Sci 1994;38:43-49.

38. Urban RJ. Neuroendocrinology of aging in the male and female. Endocrinol Metab Clin North Am 1992;21:921-931.

39. Wang X, Martindale JL, Liu Y, Holbrook NJ. The cellular response to oxidative stress: influences of mitogen-activated protein kinase signaling pathways on cell survival. Biochem J 1998;333(Pt 2):291-300.

40. Ardail D, Debon A, Perret-Vivanco C, Biel-N’Garagba MC, Krantic S, Lobie PE, et al. Growth hormone internalization in mitochondria decreases respiratory chain activity. Neuroendocrinology 2010;91:16-26.