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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-nitropropionic 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. **Journal of Movement Disorders 2013;6:28-33**

Key Words: Growth hormone, 3-nitropropionic acid, Huntington's disease, Mitochondria.

Huntington's disease (HD), an autosomal dominant neurodegenerative disorder, is clinically characterized by progressive abnormalities of movement, cognitive impairment, and neuropsychiatric symptoms. With the cloning of the Huntingtin gene in 1993,[1] experimental works have improved the understanding of the pathogenesis of HD. However, advances in the treatment strategy have not been achieved, and proven medical therapy is currently unavailable. The pathogenesis of HD affects multiple levels of cellular and molecular processes, including transcription, oxidative stress, mitochondrial defects, excitotoxicity, and activation of death effector proteases.[2] Given the multiple cellular events contributing to the pathology, integrated modulation of cell death/survival pathways might be expected.[3] A defect in mitochondrial metabolism through secondarily enhancing excitotoxicity or apoptosis is one of the pathogenic mechanisms that have been proposed to lead to striatal degeneration in HD.[4-6] Mitochondrial dysfunction in complex II-III was also reported in the caudate nuclei of patients with HD.[4,7] Thus, administration of 3-nitropropionic acid (3NP) can inhibit the mitochondrial succinate dehydrogenase-complex II[4,5,7,8] and induces striatal degeneration that resembles the pathology of the HD brain.[9,10] The progression of HD is also correlated with the aging phenomenon. The length of the CAG/polyglutamine repeat is inversely correlated with the age of onset, suggesting that neurodegeneration occurs in an aging-dependent manner.[11] Transcriptional changes, down-regulation of genes involved in synaptic function, vesicular transport, calcium

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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 zoletile hydrochloride (Sigma, 90 mg/kg). An incision was made below the base of the neck, and an Alzet osmotic minipump (flow rate 10 ul/hr, model 2ML1; Alzet, Palo Alto, CA, USA) containing 3NP was positioned under the skin. 3NP was dissolved in 0.1M phosphate-buffered saline (PBS, pH 7.4) and then adjusted to pH 7.3-7.4 with 5N NaOH. The final concentration of 3NP in the minipump was adjusted according to rat weight on the day of implantation to deliver 63 mg/kg/day.

All procedures were approved by the Clinical Research Institute, Seoul National University Hospital, and have been complied with the Guide for the Care and Use of Laboratory Animals.

GH treatment

For the treatment with GH, 2.0 IU of human recombinant GH was dissolved in 0.5 mL of PBS. For rats in the 3NP + GH group, intraperitoneal injection at the dosage of 0.3 IU/kg/day was given. The first dose was administered two hours following the osmotic minipump implantation (day 0), and treatment was then repeated everyday for five days (day 5). In the control group, PBS (0.5 mL) was administered to rats during the same period. In the cell culture, GH was incubated 30 minuets before the treatment of 3NP.

Neurological scale

Neurologic impairment scores were measured daily throughout the experiment, as previously described.[19,20] The behavioral test scores were determined by degree of abnormalities, such as recumbency (0, 1, 2), dystonia of hind legs (0, 1, 2, 3), gait (0, 1), balance on a platform (0, 1), and grasping (0, 1). A total score of 8 points indicated maximal neurological deficit, and a score of 0 points denoted normal performance.

Cell culture

The mouse neuroblastoma cell line (N18TG2) was maintained in Dulbecco's modified Eagles's medium/F-12 growth medium (Gibco, Grand Island, NY, USA) with Sato's components (Sigma, St. Louis, MD, USA) and 2% heat-inactivated newborn calf serum (HyClone, Logan, UT, USA). Cells were grown in log-phase growth on poly-(L-ornithine)-precoated culture dishes (Falcon, Franklin Lakes, NJ, USA). After confluency, the cells were plated in 96-well plates (NUNC, Denmark) at a density of 1×10^4 cells per well.

MTT assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cell viability and reflect mitochondrial activity, since tetrazolium is reduced to formazan by mitochondrial dehydrogenase activity. Following the incubation of MTT (5 mg/mL in PBS, Amresco, Solon, OH, USA) for three hours, the solution was removed, and the formazan precipitate was dissolved in 200 μL dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany). The spectrophotometric measure of absorbance was checked at 540 nm, using DMSO as a blank.

Statistical analysis

All data in this study are presented as the means ± standard deviations. Error bars in the figures are standard errors of the means. Mann-Whitney U test was used for inter-group comparisons. A two-tailed probability value of < 0.05 was considered to be significant.

Results

GH increased neurologic deficits

Loss of Nissl (+) cells in the striatum was confirmed with 3NP. Mild degree of striatal injury was observed when 3NP was injected at 56 mg/kg/day. When 3NP was injected at 66 mg/kg/day, there was more striatal damage with a lower functional motor score (Figure 1).

Until day 2, there were no differences in the neurologic scales between the 3NP + GH group and the 3NP + vehicle group. Both groups showed a test score of 0 points, indicating no deficit, and the functional status was the same as normal. From day 3, rats in the GH group began to show higher scores than those of the control group. At day 4, the deficits progressed, and then three of the rats treated with GH died be-

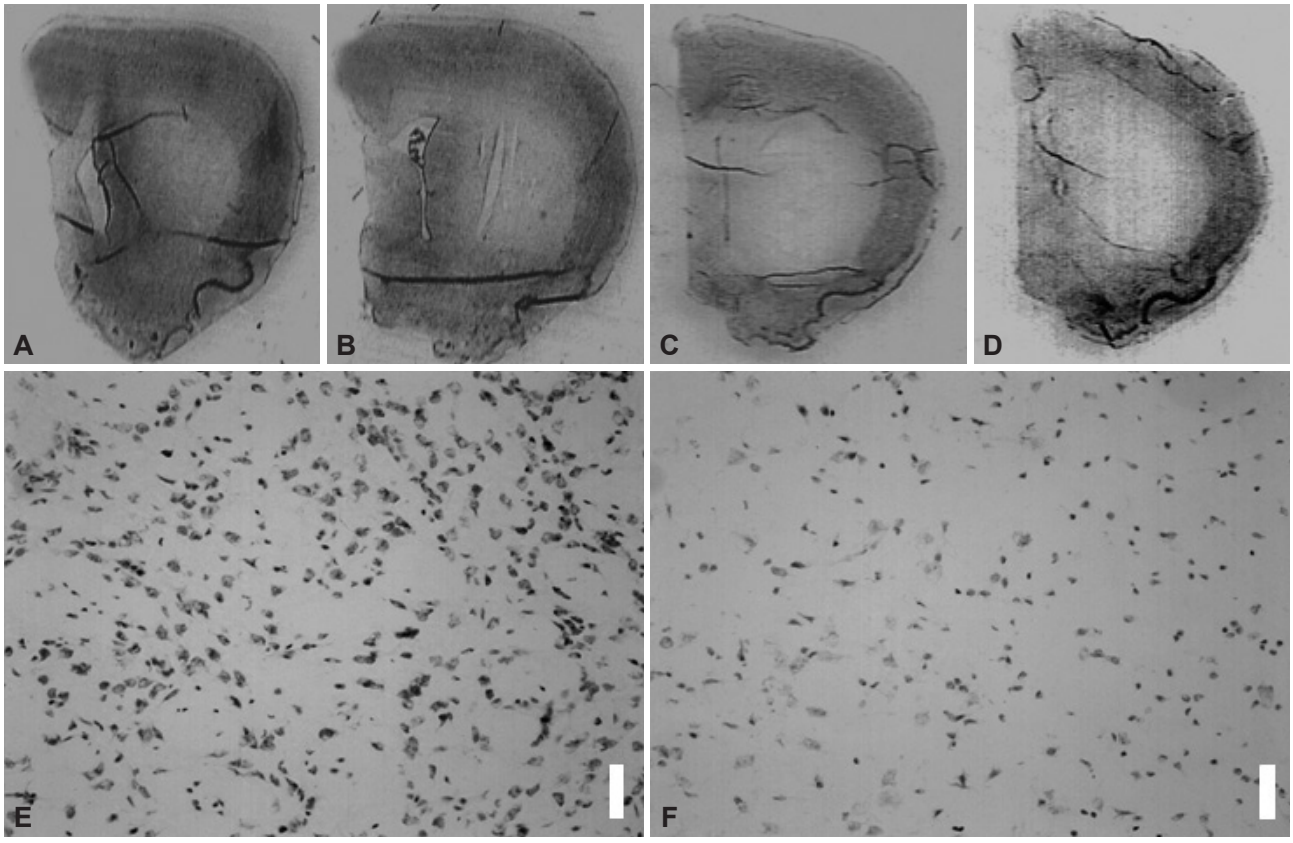

Figure 1. 3-nitropropionic acid (3NP) induced striatal degeneration. A: Control. B: Mild degree of striatal injury when 3NP was injected at 56 mg/kg/day. The neurological motor score was 2 in this case. C: When 3NP was injected at 66 mg/kg/day, the lesion showed more damage, with a functional motor score of 6. D: Severe degree of striatal injury with motor score of 8 at 66 mg/kg/day. F: Loss of Nissl (+) cells in the striatum when compared to that of the control (E) (bar = 50 μm).

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.[21] 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

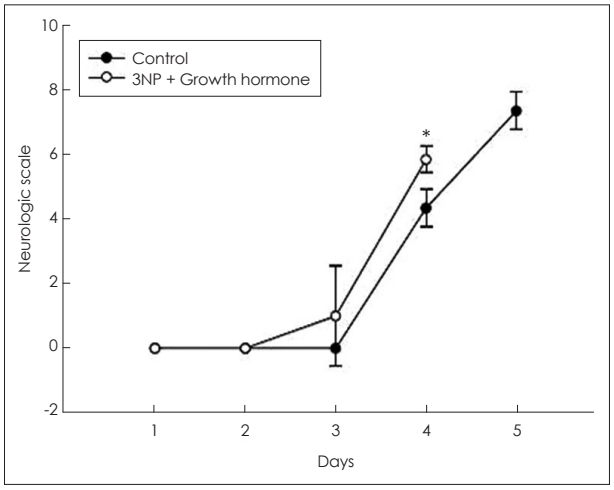

Figure 2. Growth hormone (GH) deteriorates neurological score compared to the treatment with only 3-nitropropionic acid (3NP). The neurological scores were evaluated everyday. Until day 2, the two groups (with or without GH treatment) were not different and both remained at a total score of 0. However, at day 3, rats began to deteriorate, with a more worsening trend in the GH-treated group. At day 4, GH-treated rats were significantly different from those in the control group and were in a more disabled state, with a mean score of 6 (*< 0.05, by Mann-Whitney nonparametric test). At day 5, unfortunately, the rats in the GH treated group were not able to survive (n = 6).

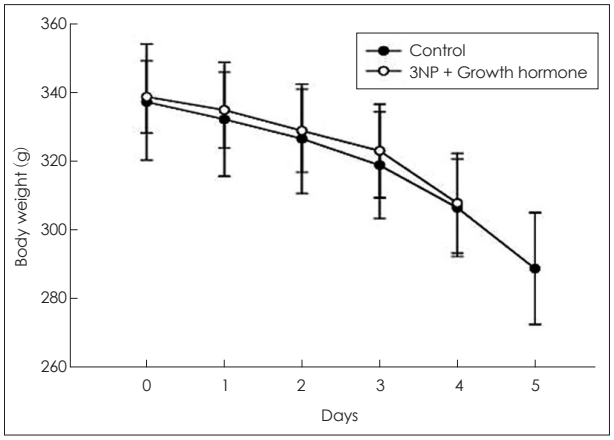

Figure 3. The effect of Growth hormone (GH) on body weight in 3-nitropropionic acid (3NP) model. With the application of 3NP, body weight usually declined. The GH treatment group also showed a decrease of body weight, which is not different from that of the control group.

fatty acids, or[22] 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,

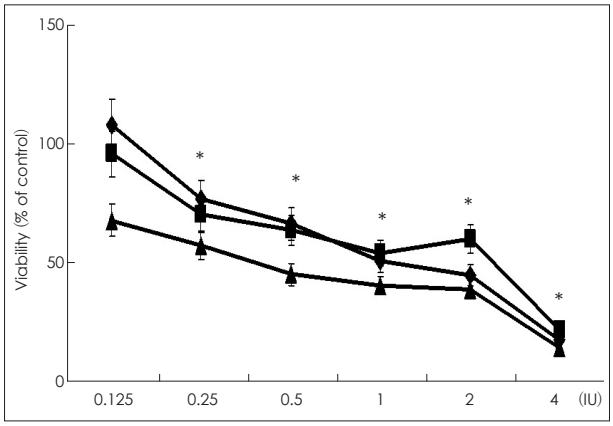

Figure 4. Mitochondrial viability assay according to the dose of Growth hormone (GH). When compared to control (100%), the relative absorbance ratios of the 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay were plotted in three different independent experimental trials. When the GH concentration was 0.25 IU/mL or higher, the % of mitochondrial activity or cell number decreased in a dose dependent pattern.

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 differentiation[21,28,29] 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.[6] 3NP was used to develop an HD model,[9] and complex II mitochondrial dysfunction can comprise the specific changes found in the brains of HD.[4,6,7,32] 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 internalized in the mitochondria that decrease respiratory chain activity.[40] 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 experiment, 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.

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