HIGHLIGHTS

The neuroprotective effects of human growth hormone as a potential treatment for amyotrophic lateral sclerosis

Human growth hormone (GH) is a single-chain polypeptide of 191 amino acids that is involved in the regulation of various physiological processes, such as growth and metabolism. GH is synthesized and secreted by somatotropic cells in the anterior pituitary gland. Its secretion is primarily regulated by a balance between growth hormone-releasing hormone and growth hormone-inhibiting hormone, which are released from the hypothalamus. In addition, GH secretion can be stimulated by ghrelin, estrogen, levodopa (L-DOPA), exercise, and fasting, and inhibited by hyperglycemia, glucocorticoids, and dihydrotestosterone. Synthetic GH can be produced with recombinant DNA technology, and is referred to as somatropin. Somatropin is used for the treatment of GH deficiency in children and adults, and has replaced endogenous GH obtained from human cadavers.

GH binds to receptors within the cell membrane and directly stimulates the proliferation of target cells, such as adipocytes and chondrocytes. GH can also act indirectly through the synthesis of insulin-like growth factor 1 (IGF-1) in the liver and target tissues. IGF-1 exerts its growth-promoting effects in a wide range of tissues, leading to bone and muscle growth. With regard to metabolism regulation, GH promotes protein anabolism, mobilization of stored triglycerides, hepatic glucose production, and insulin resistance (Vijayakumar et al., 2010). Besides promoting growth and metabolism peripherally, GH has significant effects in the brain as well. For example, GH treatments can enhance cognitive function, provide neuroprotection, and increase neurogenesis (Aberg et al., 2000; Azcoitia et al., 2005; Nyberg and Hallberg, 2013).

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease characterized by the progressive degeneration of upper and lower motor neurons. The pathophysiology of ALS can involve several factors, such as oxidative stress, excitotoxicity, and impaired energy metabolism. Currently, there are no treatments available to slow down disease progression or stop neurodegeneration. GH insufficiency has been noted in ALS and GH has neuroprotective effects; therefore, we investigated the therapeutic efficacy of GH replacement in ALS and recently reported that GH treatment was neuroprotective in cellular and animal models of ALS (Chung et al., 2015). This paper describes GH-related signal transduction pathways, its physiological functions in the brain, and its neuroprotective effects, with a focus on the therapeutic potential of GH in ALS.

Growth hormone signaling pathways: The GH receptor is a transmembrane protein that belongs to the cytokine/hematopoietic receptor superfamily. One molecule of GH binds to two GH receptors, which leads to receptor dimerization and janus kinase 2 (JAK2) tyrosine kinase activation. In turn, JAK2 autophosphorylates as well as phosphorylates GH receptors at tyrosine residues, which serve as binding domains for downstream signaling molecules. GH signal transduction pathways involve a series of signaling molecules that are described in more detail as below (Herrington and Carter-Su, 2001).

The JAK-STAT pathway: Signal transducer and activator of transcription (STAT) proteins are important transcription factors in JAK2-dependent GH pathways. Phosphorylated STAT proteins form homo- or heterodimers, and subsequently move into the nucleus to regulate the transcription of genes associated with the biological functions of GH. GH-regulated genes include serine protease inhibitor 2.1, insulin, the acid labile subunit, and CYP3A10. These genes contain promoters with STAT-binding sites, such as the interferon-γ activated sequence-like element (Herrington et al., 2000). Genetic disruption of STAT5 can impair STAT5 phosphorylation and translocation, in conjunction with growth retardation and defective IGF-1 expression. These results demonstrate the importance of STAT5-mediated gene regulation and its association with growth and metabolism.

The MAPK pathway: The mitogen-activated protein kinase (MAPK) pathway plays a crucial role in the GH signaling mechanism. In an *in vitro* study, GH treatment led to neuronal proliferation along with increased ERK phosphorylation; GH-induced proliferation was inhibited by MAPK inhibitor (PD98050) treatment (Lyuh et al., 2007). MAPK is activated by JAK2-mediated phosphorylation through the Src homology 2 domain containing (SHC) proteins. However, several lines of evidence have demonstrated the presence of a JAK2-independent activation of MAPK, which is mediated by Src family kinases. c-Fos, a proto-oncogene, is a well-known target regulated by GH through the MAPK pathway. MAPK plays a crucial role in phosphorylating Elk-1, which in turn, mediates the transcriptional activation of the serum response element, an enhancer of the *c-fos* gene (Hodge et al., 1998).

The PI3K pathway: In addition to the MAPK pathway, the phosphatidylinositol-3-kinase (PI3K) pathway is also activated by GH. Insulin receptor substrates (IRS) adaptor proteins can be phosphorylated by GH, which then interact with PI3K. PI3K activation is involved in glucose transport, lipid metabolism, cell proliferation, and cell survival. The PI3K-dependent actions of GH are mediated by the serine/threonine kinase Akt, which exerts a pivotal role in glucose metabolism, anti-apoptosis, and cell proliferation *via* glucose transporter 4 (GLUT4) translocation and glycogen synthase kinase 3 (GSK3) phosphorylation (Zhu et al., 2001).

The effects of GH in the brain: It is well established that GH has profound effects on the central nervous system (CNS). During neural development, GH is involved in neurogenesis, myelination, and synaptogenesis. GH is also implicated in adult brain functions, including learning and memory, locomotion, psychological behaviors, and neuroprotection. GH receptors and IGF-1 receptors are expressed in various brain regions, such as the cerebral cortex, hippocampus, and choroid plexus. Furthermore, accumulating evidence indicates that GH and IGF-1 molecules in the peripheral blood system can be transported to the CNS through the blood-brain barrier (BBB) and blood-CSF barrier (Aberg et al., 2006). Although the pituitary gland and liver are the major sources of circulating GH and IGF-1, respectively, local synthesis of GH and IGF-1 have also been identified within the brain. Together, these findings support the action of GH in the CNS.

Among the diverse effects of GH in the brain, neuroprotection has attracted considerable attention from researchers. The neuroprotective actions of GH are implicated in a variety of

diseases, such as hypoxic-ischemic encephalopathy, cerebral infarction, traumatic brain injury, and degenerative diseases (Arce et al., 2013). However, the exact mechanism by which GH preserves neuronal integrity after injury is not fully understood. However, neuronal proliferation, differentiation, and survival, as well as the regulation of energy metabolism, may play major roles in neuroprotection. The signaling mechanisms of GH-induced neuroprotection are thought to involve the PI3K and MAPK pathways. PI3K leads to Akt phosphorylation, which exerts its survival-promoting functions *via* the inhibition of pro-apoptotic factors, such as caspase 3, 9, and GSK3β. In addition, Akt facilitates cell cycle progression through the activation of mammalian target of rapamycin (mTOR) and suppression of GSK3β. The MAPK pathway promotes neuronal survival by deactivating Bcl-2-associated death promoter (BAD), a pro-apoptotic protein, as well as by enhancing the transcription of CREB-mediated pro-survival genes. The survival promoting aspects of the MAPK pathway are well documented in studies that demonstrate ERK activation prevents cell death due to hypoxia, oxidative stress, and cytotoxic agents. Neuroprotection induced by exogenous GH administration has been extensively studied for hypoxic brain injury. Intermittent hypoxia in rats induced neuronal apoptosis in the hippocampus and consequently, cognitive dysfunction; GH treatment attenuated cleaved caspase 3 expression (a marker of neuronal apoptosis) as well as ameliorated the neurobehavioral deficits caused by hypoxia (Li et al., 2011). Exogenous GH therapy also increased levels of IGF-1, erythropoietin, and vascular endothelial growth factor, all of which have protective roles in hypoxic-ischemic brain injury. Furthermore, neuroprotection induced by GH was associated with the downregulation of apoptosis-promoting genes, including BAD and Bax (Shin et al., 2004). Taken together, these data suggest that GH may protect the brain from hypoxic insult by attenuating neuronal apoptosis.

In addition to neuroprotection, GH promotes neurogenesis. GH regulates the proliferation and differentiation of neural stem cells in the major neurogenic regions of the brain, such as the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus. Recombinant human GH promoted the proliferation of neural stem cells as well as neural progenitors isolated from human fetal cortices (Pathipati et al., 2011). Similar to GH, IGF-1 administration also induced the proliferation of neural progenitors in the dentate gyrus in adult rats, as assessed by an increased number of BrdU-positive cells (Aberg et al., 2000). However, cellular responses to GH seem to vary with GH dose. GH can induce neuronal proliferation or differentiation in a dose-dependent manner *in vitro*: low doses of GH induced cellular proliferation, while higher concentrations of GH induced cellular differentiation along with neurite outgrowth (Lyuh et al., 2007). The proliferative effects of GH are mediated by MAPK activation, which prevents neuronal apoptosis. Neuronal differentiation induced by high doses of GH was associated with nuclear fragmentation and poly-ADP-ribose polymerase (PARP) cleavage, suggesting that programmed cell death followed the differentiation process. These findings warrant further investigation to determine the appropriate GH doses for therapeutic application.

Neuroprotection in ALS: ALS is a neurodegenerative disorder that causes progressive motor weakness, skeletal muscle atrophy, and eventual death, with a median survival of 3 to 5 years. This disease is characterized by the selective loss of motor neurons in the motor cortex, brain stem, and anterior horn of the spinal cord. The cellular mechanisms underlying neurodegeneration in ALS include glutamate excitotoxicity, oxidative damage, abnormal protein aggregation, mitochondrial impairment, and neuroinflammation. However, knowledge about the key components of ALS pathophysiology is still limited. The only available treatment is riluzole, but this drug improves survival only by a few months and delays the onset of ventilator support in a select number of subjects. Researchers are still seeking to understand the mechanisms of ALS neurodegeneration more fully, and to develop more effective therapeutics based on ALS patho-mechanisms.

Impaired GH secretion has been reported in approximately two-thirds of individuals with ALS. Transgenic mouse models of ALS recapitulate certain aspects of the disease, including an impairment in the somatotropic axis. Since GH has neuroprotective effects in several neurological diseases, and GH hormone insufficiency has been noted in ALS, GH replacement therapy is being considered as a possible ALS treatment. Our recent study demonstrated the protective effects of GH in *in vitro* and *in vivo* models of familial ALS (Chung et al., 2015). Copper/zinc (Cu/Zn) superoxide dismutase (SOD1) gene mutations are responsible for 20% of familial ALS worldwide. Two mutant cell lines have been established by transfecting two different human SOD1 cDNA sequences into the ventral spinal cord 4.1 (VSC4.1) hybrid cell line: A4V (alanine at codon 4 substituted to valine) and G93A (glycine at codon 93 substituted to alanine). GH pre-treatment mitigated homocysteine-induced neuronal cytotoxicity in the mutant cell lines. However, Bax expression and PARP cleavage were not significantly changed, suggesting that the effects of GH may be mediated by a non-mitochondrial pathway. In addition, we confirmed the *in vivo* effects of GH treatment in transgenic mice that carried the SOD1G93A mutation. GH administration significantly improved their motor performance, weight loss, and life span compared to saline-treated control mice. In addition, GH treatment prevented motoneuronal loss and astrogliosis induced by the SOD1 mutation. Moreover, Bcl-2 expression was restored in spinal motor neurons, indicating that GH inhibited apoptotic cell death.

Growing evidence suggests that GH and IGF-1 supplementation provide neuroprotection in ALS models, as demonstrated by our results. Viral delivery of IGF-1 delayed disease onset, as well as improved the mortality of the transgenic mice. Effects of IGF were greater in magnitude than the improvements seen with glial cell line-derived neurotrophic factor (GDNF). The enhanced function of the somatotropic axis in combination with physical activity revealed substantial, synergistic effects on neuronal viability, motor function improvement, and survival benefit (Kaspar et al., 2005). IGF-1 was delivered more effectively into the CNS by using viral vectors that targeted the ventricular system. This study demonstrated that more efficient IGF-1 delivery led to the upregulation of trophic factors throughout the brain and a significant improvement in motor function (Dodge et al., 2010). The neuroprotective effects of IGF-1 were also confirmed *in vitro* with increased expression of phosphorylated Akt.

Although the beneficial effects of GH and IGF-1 supplementation are well-established in cellular and animal models, clinical trials with GH in ALS patients have failed to demonstrate significant improvements in neuronal death, mortality, or disease progression (Sacca et al., 2012). The negative ALS clinical trial results with GH may be attributed to multiple factors,

including inappropriate GH doses, GH resistance, stage-specific alterations in the cellular response to GH, and pathophysiologic differences between species (*e.g.*, human and mouse). Among these, GH resistance in individuals with ALS may be a crucial issue, but the molecular mechanism and its relationship with disease progression have not been thoroughly investigated. If the critical factors underlying GH sensitivity in ALS can be understood, the efficacy of GH treatment can be improved. A recent study investigating the temporal profiles of GH secretion in ALS mice demonstrated that GH levels are paradoxically increased at symptom onset, while motor neuron death occurs during the pre-symptomatic period when GH secretion is unaltered (Steyn et al., 2013). Although compensatory GH over-production was associated with an increase in muscle IGF-1 levels, it did not affect the number of motor neurons. These findings suggest that disease-triggering factors other than GH-mediated alterations are present, and that GH alone may be insufficient to modify ALS progression. There are still many unanswered questions regarding GH function in terms of ALS. Further investigation is required to narrow the gap between bench and bedside research in order to identify future ALS therapies.

Conclusion: The neuroprotective effects of GH have been described in several *in vitro* studies, including the improvements in survival and functional outcome after GH treatment in ALS mouse models. It is likely that GH promotes neuronal survival primarily through its anti-apoptotic properties. However, clinical trials have failed to demonstrate its therapeutic potential, and there are still unresolved questions about the key mechanisms of ALS. More research is needed to consolidate the evidence of GH-mediated neuroprotection, and to investigate the factors contributing to the negative results of GH-based clinical studies for ALS.

This work was supported by grants from the Korea Health 21 R & D Project (HI14C2348) and the National Research Foundation of Korea (NRF2014R1A2A1A11051520).

Jin-Young Chung[#], Jun-Sang Sunwoo[#], Min-Wook Kim, Manho Kim[*]

Department of Veterinary Internal Medicine and Geriatrics, Kangwon National University, Gangwondo, South Korea (Chung JY)
Department of Neurology, Seoul National University Hospital, Seoul, South Korea (Sunwoo JS, Kim M)
Department of Rehabilitation Medicine, College of Medicine, The Catholic University of Korea, Seoul, South Korea (Kim MW)
Institute of Catholic Integrative Medicine (ICIM), Incheon St. Mary's Hospital, Incheon, South Korea (Kim MW)
Protein Metabolism Medical Research Center, Seoul National University College of Medicine, Seoul, South Korea (Kim M)

***Correspondence to:** Manho Kim, M.D., Ph.D., kimmanho@snu.ac.kr.*
#These authors contributed equally to this work.
***Accepted:** 2015-05-20*
***doi:**10.4103/1673-5374.162690* ***http**://www.nrronline.org/*
Chung JY, Sunwoo JS, Kim MW, Kim M (2015) The neuroprotective effects of human growth hormone as a potential treatment for amyotrophic lateral sclerosis. Neural Regen Res 10(8):1201-1203.

References

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS (2000) Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 20:2896-2903.
Aberg ND, Brywe KG, Isgaard J (2006) Aspects of growth hormone and insulin-like growth factor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain. ScientificWorldJournal 6:53-80.
Arce VM, Devesa P, Devesa J (2013) Role of growth hormone (GH) in the treatment on neural diseases: from neuroprotection to neural repair. Neurosci Res 76:179-186.
Azcoitia I, Perez-Martin M, Salazar V, Castillo C, Ariznavarreta C, Garcia-Segura LM, Tresguerres JA (2005) Growth hormone prevents neuronal loss in the aged rat hippocampus. Neurobiol Aging 26:697-703.
Chung JY, Kim HJ, Kim M (2015) The protective effect of growth hormone on Cu/Zn superoxide dismutase-mutant motor neurons. BMC Neurosci 16:1.
Dodge JC, Treleaven CM, Fidler JA, Hester M, Haidet A, Handy C, Rao M, Eagle A, Matthews JC, Taksir TV, Cheng SH, Shihabuddin LS, Kaspar BK (2010) AAV4-mediated expression of IGF-1 and VEGF within cellular components of the ventricular system improves survival outcome in familial ALS mice. Mol Ther 18:2075-2084.
Herrington J, Carter-Su C (2001) Signaling pathways activated by the growth hormone receptor. Trends Endocrinol Metab 12:252-257.
Herrington J, Smit LS, Schwartz J, Carter-Su C (2000) The role of STAT proteins in growth hormone signaling. Oncogene 19:2585-2597.
Hodge C, Liao J, Stofega M, Guan K, Carter-Su C, Schwartz J (1998) Growth hormone stimulates phosphorylation and activation of elk-1 and expression of c-fos, egr-1, and junB through activation of extracellular signal-regulated kinases 1 and 2. J Biol Chem 273:31327-31336.
Kaspar BK, Frost LM, Christian L, Umapathi P, Gage FH (2005) Synergy of insulin-like growth factor-1 and exercise in amyotrophic lateral sclerosis. Ann Neurol 57:649-655.
Li RC, Guo SZ, Raccurt M, Moudilou E, Morel G, Brittian KR, Gozal D (2011) Exogenous growth hormone attenuates cognitive deficits induced by intermittent hypoxia in rats. Neuroscience 196:237-250.
Lyuh E, Kim HJ, Kim M, Lee JK, Park KS, Yoo KY, Lee KW, Ahn YO (2007) Dose-specific or dose-dependent effect of growth hormone treatment on the proliferation and differentiation of cultured neuronal cells. Growth Horm IGF Res 17:315-322.
Nyberg F, Hallberg M (2013) Growth hormone and cognitive function. Nat Rev Endocrinol 9:357-365.
Pathipati P, Gorba T, Scheepens A, Goffin V, Sun Y, Fraser M (2011) Growth hormone and prolactin regulate human neural stem cell regenerative activity. Neuroscience 190:409-427.
Sacca F, Quarantelli M, Rinaldi C, Tucci T, Piro R, Perrotta G, Carotenuto B, Marsili A, Palma V, De Michele G, Brunetti A, Brescia Morra V, Filla A, Salvatore M (2012) A randomized controlled clinical trial of growth hormone in amyotrophic lateral sclerosis: clinical, neuroimaging, and hormonal results. J Neurol 259:132-138.
Shin DH, Lee E, Kim JW, Kwon BS, Jung MK, Jee YH, Kim J, Bae SR, Chang YP (2004) Protective effect of growth hormone on neuronal apoptosis after hypoxia–ischemia in the neonatal rat brain. Neurosci Lett 354:64-68.
Steyn FJ, Lee K, Fogarty MJ, Veldhuis JD, McCombe PA, Bellingham MC, Ngo ST, Chen C (2013) Growth hormone secretion is correlated with neuromuscular innervation rather than motor neuron number in early-symptomatic male amyotrophic lateral sclerosis mice. Endocrinology 154:4695-4706.
Vijayakumar A, Novosyadlyy R, Wu Y, Yakar S, LeRoith D (2010) Biological effects of growth hormone on carbohydrate and lipid metabolism. Growth Horm IGF Res 20:1-7.
Zhu T, Goh EL, Graichen R, Ling L, Lobie PE (2001) Signal transduction via the growth hormone receptor. Cell Signal 13:599-616.