AREA:
Ricerca farmacologica di base e clinica

# Selected Growth Hormone Secretagogues (GHS) decrease mutant SOD1 toxicity in an in vitro model of amyotrophic lateral sclerosis

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Background:
Amyotrophic lateral sclerosis (ALS) is a motor neuron disease characterized by progressive degeneration of upper and lower motor neurons, resulting in muscle atrophy, limb paralysis, and finally respiratory failure. ALS pathogenetic mechanisms are still unclear even though (i) mutations of superoxide dismutase 1 (SOD1) and (ii) increased oxidative stress have been linked with several variants of ALS. SOD1 is an antioxidant enzyme, whose substitution of glycine 93 to alanine (SOD1-G93A) is a mutation present in about 20% of familial and 5% of sporadic ALS, and leads to gain/loss of function that enhances the accumulation of highly toxic hydroxyl radicals.
The current standard of care involves riluzole and edaravone, while all the other interventions are only symptomatic and palliative. Therefore, there is a strong need to characterise more effective drug.
GHS are a large family of synthetic compounds which have shown endocrine functions, through the stimulation of growth hormone (GH) release, and extra-endocrine properties, including stimulation of food intake and lean mass, at least in part by the binding to GHS-R1a, the receptor of ghrelin.
Among GHS, we have investigated the effects of (i) hexarelin, which has important neuroprotective and cytoprotective activities, both in vitro and in vivo; and (ii) JMV2894, which stimulates Ca2+ mobilization in vitro and GH release in vivo, and modulates mitochondria functioning and ROS production.
Methods:
SH-SY5Y SOD1-G93A cells, a human neuroblastoma cell line that expresses SOD1-G93A enzyme, were treated with hydrogen peroxide (H2O2) and GHS to study the protective effect of GHS against increased oxidative stress. Photomicrographs of stained cells were quantified by skeleton and fractal analysis. The mRNA expression levels of caspase 3, caspase 7, Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) were quantified by real-time PCR, while the protein levels of mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-

kinase (PI3K)/protein kinase B (Akt), and histone H2AX phosphorylated at Ser139 (γH2AX) were measured by western blot.

Results:

The treatment of SH-SY5Y SOD1-G93A cells with $H_2O_2$ induces important changes in cell morphology which can be antagonize by hexarelin and JMV2894 incubation.

In addition, hexarelin exerts anti-apoptotic effects by modulating the mRNA levels of proteins belonging to the BCL-2 family as well as the activation of effector caspases.

The protective effects of hexarelin and JMV2894 are mediated by the activation of molecules that regulate apoptosis, promoting cell survival processes.

Conclusions:

Hexarelin and JMV2894 are capable of protecting cells from $H_2O_2$-caused cytotoxicity, suggesting the possibility of developing new anti-oxidant and neuroprotective drugs with improved therapeutic potential. Further investigations are required to (i) clarify GHS molecular mechanisms of action, and (ii) whether their effects are mediated by GHS-R1a.

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