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Title:

Selected Growth Hormone Secretagogues (GHS) as promising therapeutical approach for Amyotrophic Lateral Sclerosis: a proof-of-concept study

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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. The mutation in SOD1 with the replacement of glycine 93 by alanine is present in about 20% of familial and 5% of sporadic ALS, and is responsible for a conformational change that leads to a gain of function resulting in oxidative stress, mitochondrial alterations, and apoptosis.

The current standard of care involves riluzole, edaravone and tofersen, but none of these treatments have proved curative. Therefore, there is a strong need to characterise more effective drugs.

Growth hormone secretagogues (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.

This research aims to investigate the potential protective effects of two selected GHS, hexarelin and JMV2894, against massive oxidative stress conditions. Hexarelin has important neuroprotective and cytoprotective activities, both in vitro and in vivo; JMV2894 stimulates Ca^{2+} mobilization, modulates mitochondria functioning and ROS production in vitro, and GH-release in vivo.

To study the neuroprotective effects of hexarelin and JMV2894, human neuroblastoma cells overexpressing SOD1G93A enzyme were incubated for 24h with 150µM H_2O_2 or with the combination of H_2O_2 and 1µM hexarelin or 1µM JMV2894.

Morphometric quantification showed that H_2O_2-treatment induced an apoptotic phenotype that was rescued by both GHS. The quantification of mRNA levels of the BCL-2 family and those of the effector caspase proteins suggest that GHS have anti-apoptotic effects: both GHS significantly decreased Bax/Bcl-2 ratio and hexarelin also inhibited the activation of caspase-3.

The molecular pathways involved in GHS neuroprotection include the modulation of MAPKs and PI3K/Akt phosphorylation, probably through epigenetic mediation. Immunofluorescence visualization of γH2AX nuclear foci showed that hexarelin and JMV2894 significantly decreased the percentage of γH2AX-positive cells compared to the H_2O_2-treated group.

Moreover, GHS were found to reduce the inflammatory state and mitochondrial damage caused by the mutation by significantly reducing the mRNA expression levels of TNFα, Sesn2 and MMP2, markers also related to the autophagy mechanism.

The results suggest that developing new GHS-based anti-oxidant and neuroprotective drugs with improved therapeutic potential may be useful in ALS therapy, even though further investigations are required to clarify GHS molecular mechanisms of action.