RESVERATROL TREATMENT REDUCES THE VULNERABILITY OF SH-SY5Y CELLS AND CORTICAL NEURONS OVEREXPRESSING SOD1-G93A TO THIMEROSAL TOXICITY THROUGH SIRT1/DREAM/PDYN PATHWAY

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Introduction: In humans, mutation of glycine 93to alanine of Cu^{++}/Zn^{++} superoxide dismutase type-1(SOD1-G93A) has been associated to some familial cases of Amyotrophic Lateral Sclerosis (ALS). Several evidence proposed the involvement of environmental pollutants that like mercury could accelerate ALS symptoms. SH-SY5Y cells stably transfected with SOD1and G93A mutant of SOD1constructs were exposed to non-toxic concentrations (0.01μM) of ethylmercury thiosalicylate (thimerosal) for 24h.

Materials and methods: SH-SY5Y and cortical neurons were treated with MeHg (0.1μM) and Thim (0.01μM). For stable transfection of SH-SY5Y cells, were used the following constructs: (1) pcDNA3.1, (2) pF151pcDNA3.1(+)SOD1WT and (3) pF155pcDNA3.1(+)SOD1-G93, whereas transient cell transfection was performed with anti-sense (AS) and missense (MS) oligonucleotides (ODNs) against human SIRT1. qRT-PCR and Western blotting experiments were performed to analyze mRNA and protein levels. Immunoprecipitation assay were achieved to verify the levels of DREAM ubiquitination and deacetylation after Thim exposure,

Results: Our results demonstrated that the thimerosal, in SOD1-G93A cells, but not in SOD1cells, reduced cell survival. Furthermore, thimerosal-induced cell death occurred in a concentration dependent-manner and was prevented by the Sirtuin 1(SIRT1) activator Resveratrol (RSV). Moreover, thimerosal decreased the protein expression of transcription factor Downstream Regulatory Element Antagonist Modulator (DREAM), but not DREAM gene. Interestingly, DREAM reduction was blocked by co-treatment with RSV, suggesting the participation of SIRT1in determining this e ect. Immunoprecipitation experiments in SOD1-G93A cells exposed to thimerosal demonstrated that RSV increased DREAM deacetylation and reduced its polyubiquitination. In addition, RSV counteracted thimerosal-enhanced prodynorphin (PDYN) mRNA, a DREAM target gene. Furthermore, cortical neurons transiently transfected with SOD1-G93A construct and exposed to thimerosal (0.5μM/24h) showed a reduction of DREAM and an up-regulation of the prody- norphin gene. Importantly, both the treatment with RSV or the transfection of siRNA against prodynorphin significantly reduced thimerosal-induced neurotoxicity, while DREAM knocking-down potentiated thimerosal- reduced cell survival.

Discussion and conclusions: Collectively, our results demonstrate that neuronal cells expressing human SOD1carrying G93A mutation are more susceptible to Thim toxicity, through SIRT1/DREAM/PDYN pathway, and indicate a possible role of this neurotoxicant in the worsening of ALS in genetically vulnerable organisms.