Methylphenidate and Amphetamine did not change neurite outgrowth in undifferentiated SH-SY5Y neuronal cells

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Introduction: According to the Diagnostic And Statistical Manual Of Mental Disorders–5 (DSM-5), attention-deficit/hyperactivity disorder (ADHD) is “a persistent pattern of inattention and/or hyperactivity-impulsivity interfering in functioning or development, as characterized by inattention and/or hyperactivity and impulsivity” [1], globally affecting 5%–7.2% of youth and 2.5%–6.7% of adults [2]. The treatment mostly focus on symptoms. Methylphenidate (MPH) and amphetamine (AMPH), are well-known psychostimulants for their ability to promote monoamine levels increase [3] used in ADHD [4]. Herein, we evaluated AMPH and MPH potential to promote neurite outgrowth and synaptogenesis in undifferentiated SH-SY5Y neuronal cells.

Material and Methods: Unifferentiated SH-SY5Y neuronal human cells were exposed to clinically relevant concentrations of either MPH or AMPH for 96 hours to evaluate their cytotoxicity through MTT reduction assay. Thereafter, neurite outgrowth and synaptogenesis, were evaluated through Western blotting, using the higher cellular density (25000 cm^2) and microphotographs analysis to count neurites using NeuronJ software at the lower cellular density (6250 cells/cm^2). To validate the experimental protocol, we evaluated the neurite outgrowth of SH-SY5Y after 96 hours of exposure to β-NGF as a positive control. Statistical analysis was conducted using GraphPad Prism. When the distribution was normal, a parametric analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test. When data did not follow a normal distribution, statistical analysis was performed using the Kruskal–Wallis test, followed by Dunn's post-hoc test when a significant p was reached. Results were considered significantly different when $p < 0.05$.

Results: Cytotoxic evaluation revealed that the second lowest concentration of AMPH induced cytotoxicity at the lower cellular density, while the lower concentration worked as cytotoxic thought the MTT assay at the higher cellular density after 96 hours of exposure. Importantly, both concentrations of β-NGF reduced cytotoxicity at both cellular densities, as expected. The expression of neuronal proteins synaptophysin, PSD95 and GAP43 were not affected by the drugs, as well as by the positive control β-NGF. Finally, no changes in neurite outgrowth was evoked by AMPH or MPH or even β-NGF.

Conclusions: As far as we know, we are conducting the first study using clinically relevant concentrations of MPH and AMPH in a paradigm of acute exposure to

evaluate the effects of neurogenesis in undifferentiated SH-SY5Y cells. More studies are needed for shorter periods of exposure attempting to reveal new data and experimental paradigms to assess the neuroprotection potential on these amphetamines.

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