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Cellular mechanisms of 1,3-DMAA-induced neurotoxicity in SH-SY5Y cells

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Abstract

Background: 1,3-Dimethylamylamine (1,3-DMAA) is a chiral sympathomimetic amine commonly added to dietary supplements marketed for weight loss, performance enhancement, and recreational purposes [2]. Despite regulatory bans, 1,3-DMAA continues to be detected in doping controls and dietary supplements, raising toxicological concerns. However, the cellular mechanisms underlying its neurotoxic potential remain incompletely characterized [3]. **Objective**: This study aimed to investigate the cytotoxic mechanisms induced by 1,3-DMAA in a human neuronal cell model (SH-SY5Y), contributing to a better understanding of its toxicodynamics. **Methods**: SH-SY5Y cells were exposed for 48 h to 1,3-DMAA (1.3×10^{-4} to 1.5×10^{1} mM; n=5); mitochondrial metabolic activity was assessed using the MTT assay and the lysosomal integrity through the neutral red uptake (NR) assay. Based on the MTT results, cells were subsequently exposed to the EC_{20} (4.21 mM), EC_{40} (4.91 mM), and EC_{60} (5.59 mM), and changes in intracellular reactive oxygen species (ROS) production and mitochondrial membrane potential ($\Delta\Psi m$) were assessed using fluorometric probes. Autophagic features were evaluated using acridine orange (AO) staining to detect acidic vesicular organelles. **Results**: 1,3-DMAA induced concentration-dependent cytotoxicity, with a greater impact on mitochondrial function than lysosomal integrity, as evidenced by lower EC_{50} values in the MTT assay compared to the NR assay (5.24 mM versus 6.36 mM, respectively). 1,3-DMAA induced a concentration-dependent increase in intracellular ROS levels from EC20 (236.67%; $p<0.001$) and EC40 (211.87%; $p<0.01$) and peaking at EC_{60} (272.05%; $p<0.0001$). In contrast, $\Delta\Psi m$ remained unchanged at lower concentrations, with a significant increase observed at EC_{60} (317.32%; $p<0.0001$). AO staining showed increased acidic vesicular organelles at higher concentrations. **Conclusions**: The concomitant increase in ROS and mitochondrial hyperpolarization of $\Delta\Psi m$ indicates a pro-oxidant state. The increase in acidic vesicular organelles suggests activation of autophagic processes and/or progression to apoptosis. These findings provide mechanistic insight into 1,3-DMAA-induced neurotoxicity and establish a foundation for further toxicological investigations.

Keywords: neurotoxicity; *in vitro* assay; SH-SY5Y cells

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