

Cellular Responses of Human Neuroblastoma Cells to 1,3-DMAA Exposure

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Background: Once used as a nasal decongestant until the 1980s, 1,3-dimethylamylamine (1,3-DMAA) was withdrawn from the market due to adverse effects such as headaches, nervousness, psychomotor stimulation, and tremors [1]. In 2005, 1,3-DMAA re-emerged on the market and is now found in dietary supplements for appetite suppression, ergogenic aids for athletic performance, and for psychoactive recreational products [2]. Despite its controversial use and ban in several competitions, its presence in anti-doping analyses and supplements continues to generate significant concern. Although the toxic effects are known, the neurotoxic impact is still not fully understood [3-4]. **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 48h 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 EC₂₀ (4.21 mM), EC₄₀ (4.91 mM), and EC₆₀ (5.59 mM), and changes in intracellular reactive oxygen species (ROS) production and mitochondrial membrane potential ($\Delta\Psi_m$) 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 EC₅₀ 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 EC₆₀ (272.05%; $p<0.0001$). In contrast, $\Delta\Psi_m$ remained unchanged at lower concentrations, with a significant increase observed at EC₆₀ (317.32%; $p<0.0001$). AO staining showed increased acidic vesicular organelles, also at higher concentrations. **Conclusions:** The concomitant increase in ROS and mitochondrial hyperpolarization indicate a pro-oxidant state. The increase in acidic vesicular organelles suggests activation of autophagic processes and/or progression toward apoptosis. These findings provide mechanistic insights 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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