Anesthetic's adverse effects on human cerebral organoids: a comprehensive study from molecules to tissue

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Introduction: Recent studies in juvenile animals and children indicate detrimental impacts of early-life general anesthesia on brain development, manifesting as memory deficits, learning disabilities, and mental health issues. However, the specific pathological alterations and mechanisms underlying these effects remain largely unexplored, partly due to the absence of a suitable human model. Our laboratory has advanced the use of induced pluripotent stem cells (iPSCs) to create 3D cerebral organoids, offering a more clinically relevant human model to investigate the neurotoxic impact of the intravenous anesthetic propofol in this study.

Methods: Human iPSCs were used to generate cerebral organoids through a sequential culturing process in a chemically defined medium. These organoids were characterized using immunostaining for neural lineage markers and electrophysiological analysis via patch clamping. At two months of maturation, organoids were exposed to a clinically relevant dose of propofol for 6 hours, either as a single exposure or triple exposures over three consecutive days. A dimethyl sulfoxide vehicle served as the control. The effects on cell apoptosis and autophagy were evaluated using caspase 3 activity assays, Western blotting, and electron microscopy. Additionally, the impact of propofol on the gene expression profile of 18,675 genes and signaling were assessed through Arraystar array analysis, with validation by real-time PCR, and bioinformatic analysis.

Results: The two-month-old cerebral organoids comprised approximately 80% neurons and 20% neural stem cells and supporting cells, including astrocytes, microglia, and oligodendrocytes. These neurons established organized synapses and exhibited functional glutamatergic and GABAergic currents. Propofol dose and exposure frequency dependently induced neurotoxicity. Specifically, exposure to propofol for 6 hours resulted in increased cleaved caspase 3 expression, indicative of neuroapoptosis. Electron microscopy showed enhanced autophagy and abnormal mitochondrial morphology following propofol exposure. Microarray analysis identified differential expression in 113 mRNAs (39 upregulated, 74 downregulated), with bioinformatics analysis suggesting 49 of these are involved in autophagy, mitochondrial stress, and neurodegeneration. Notably, 7 propofol-dysregulated synapse genes were linked to 35 nervous system development functions in both health and disorders, including calcium handling and synaptic cross-talk.

Conclusions: Our study demonstrates propofol's direct toxic effects on human brain tissue, unveiling complex pathological phenotypes and molecular mechanisms. Alterations in mRNA profiles, coupled with changes in apoptotic, autophagic and mitochondrial processes, may collectively contribute to developmental neurodegeneration. These findings underscore the potential of iPSC-derived human cerebral organoids as a valuable model for studying the neurodevelopmental consequences of propofol and other anesthetics. This approach offers critical insights for developing more effective neuroprotective strategies in pediatric anesthesia.