[N-68] Anesthesia-induced neurotoxicity in the developing retina: a window of opportunity

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Background: Anesthetic agents cause widespread apoptosis in the developing brain. Vulnerability coincides with the peak in synaptogenesis and anesthesia-induced neurodegeneration has been shown to result in loss of neurons, cognitive impairment, and behavioral abnormalities in a variety of newborn animal models. However, it is unknown if anesthesia-induced neurotoxicity occurs in humans because there is currently no modality to assess for neuronal apoptosis in vivo. The retina is unique in that it is the only portion of the central nervous system that can be directly visualized by non-invasive means. As in the brain, programmed cell death occurs naturally in the developing retina and is critical for synaptogenesis and elimination of aberrant connections. Thus, we hypothesized that anesthetics can cause neurotoxicity in the developing retina. We aimed to demonstrate that isoflurane induces apoptosis in the retina following exposure. Because high resolution non-invasive methods have been developed to image single cell apoptosis within the retina in vivo, we also tested the hypothesis that a systemically injected fluorescent probe could cross the blood-retinal barrier and bind to cells undergoing programmed cell death.
Methods: The care of the animals in this study was in accordance with NIH and Institutional Animal Care and Use Committee guidelines. 7 day old CD-1 male mouse pups underwent 1 hour exposure to isoflurane (2%) or air. Following exposure, retina was harvested and immunohistochemistry for activated caspase-3, -9, and -8 was performed. Cytochrome c release from retinal mitochondria was assessed and steady-state levels of pro- and anti-apoptotic mediators were determined with immunoblot analysis. Significance was assessed with ANOVA and post hoc Tukey’s test and significance set at $P<.05$. The types of cells undergoing apoptosis were identified with double labeling immunofluorescence. Retinal uptake and the ability of fluorescent-labeled annexin V to bind to cells undergoing natural cell death and anesthesia-induced apoptosis in the retina were determined following intraperitoneal injection.
Results: Isoflurane activated the intrinsic apoptosis pathway in the inner nuclear layer (INL) and activated both the intrinsic and extrinsic pathways in the ganglion cell layer of the retina. Immunofluorescence demonstrated that bipolar and amacrine neurons within the INL underwent physiologic cell death in air-exposed controls and were the likely targets of isoflurane-induced neurotoxicity. Following injection, fluorescent-labeled annexin V was readily detected in the INL of both air- and isoflurane-exposed mice and co-localized with activated caspase-3 positive cells.
Conclusions: These findings indicate that isoflurane-induced neurotoxicity occurs in the developing retina and lays the groundwork for development of a non-invasive imaging technique to detect anesthesia-induced neuronal apoptosis in infants and children. Thus, in future work, it may be possible to exploit neurodegeneration in the human retina as a surrogate for anesthesia-induced brain neurotoxicity.
