Inhaled Anesthesia, Apoptosis, and the Developing Retina: A Window into the Brain?

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In this month's issue of *Anesthesia & Analgesia*, Cheng et al.[1] describe how isoflurane produces apoptosis in the retina in 7-day-old mouse pups. They found that exposure to 2% isoflurane for 1 hour resulted in apoptosis in cells in the inner nuclear layer of the retina, which by double-label immunostaining appeared to be amacrine and bipolar cells. Increasingly, anesthesiologists have become concerned about the risks of exposing the immature brain to anesthetic agents. Little data are available to guide clinical practice in this regard, although animal studies have suggested that even brief exposure may not be benign[2,3]; accordingly, a means to more easily monitor for adverse effects of anesthetic exposure on neuronal cells would be a significant advance in the field. There are 2 main implications of the study by Cheng et al.: (1) isoflurane promotes apoptosis in the immature retina, perhaps in a cell-specific manner; and (2) the retina may serve as an indirect means of visualizing and understanding potentially adverse effects of inhaled anesthesia on neurons in the brain.

In recent years, it has become apparent that changes in the retina provide a "window" into the brain for a number of serious, common degenerative diseases.[4] These findings follow naturally, because the retina is formed embryologically from the central nervous system with cells and axonal structures resembling those in the brain. The retina contains layers of specialized cells from the photoreceptors to the retinal ganglion cells (RGCs) that process light. Axons from the RGCs extend to the lateral geniculate nucleus and the superior colliculus and from there to the visual cortex, where higher visual processing results in our ability to see the world.[5]

Advances in imaging have enabled detailed visualization of the retinal cell layers, axons, and retinal vasculature in humans. Examples of such techniques include ocular coherence tomography, enhanced depth imaging spectral domain ocular coherence tomography, fundus photography, and confocal scanning laser ophthalmoscopy.[6–8] Adaptive optics is another technique that compensates for aberrations in the optical path, which is used successfully to image photoreceptors in monkeys and humans.[9]

Although the direct visualization of RGCs has been used for decades in experimental models, the technology has not yet been translated for humans. Injection of tracers into the vitreous that hone in on and visualize RGCs is now feasible and undergoing clinical trials. Importantly, one of the techniques, known as DARC (detection of apoptotic retinal cells), visualizes apoptotic RGCs.[10] In theory, such techniques may allow clinicians to follow the progression of retinal diseases, including glaucoma, diabetic retinopathy, macular degeneration, and photoreceptor degeneration, among others,[11] and as a guide to treatment efficacy.

The significance of the study by Cheng et al. is that changes in the retina may be reflective of what is happening in the brain. Although a weakness of the study is that the authors did not directly compare isoflurane-induced changes in the retinal cells with those in the brain, it has been recognized for centuries that the eye may serve as a window into the brain. Intense interest has focused on the retinal manifestations of brain degenerative diseases including Alzheimer disease (AD), Parkinson disease (PD), multiple sclerosis, and vascular diseases including stroke. In particular, retinal microvascular abnormalities can predict stroke.[12] Both AD and PD cause retinal cell loss and thinning of the retinal nerve fiber layer.[8,13] τ and amyloid β proteins increase in the retina of the subjects with AD.[14] In age-related macular degeneration, a retinal disease, molecules present in drusen are also found in AD plaques.[15] RGC loss precedes axonal atrophy and disturbed axonal transport in glaucoma, a similar pattern to that of AD and PD.[16] Overall, these vascular and neurodegenerative disease studies support the translational potential for "indexing" cerebral pathology by examinations of the neural retina.

Apart from investigations of blood flow, few reports have addressed the potentially adverse effects of inhaled anesthetics on the retina.[17,18] In this study, Cheng et al. exposed 7-day-old mouse pups to 2% isoflurane or air for 1 hour. Five hours after exposure, increased terminal deoxynucleotidyl transferase-mediated UTP nick-end labeling (TUNEL) staining and caspase-3 activation (both indicators of apoptotic cells) were observed in the inner nuclear layer of the retina. Caspases-8 and -9 were increased in the RGC layer. The authors found that caspase-3 colocalized with immunostaining markers for bipolar cells and amacrine cells, both of which are found in the inner nuclear layer. Finally,

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isoflurane increased the percentage of cells in the inner nuclear layer that bound Annexin V, a phospholipid-binding protein used to detect apoptosis. Although conducted in mice, these findings may carry significant implications for our pediatric patients.

Hopefully the study by Cheng et al. will prompt further examination of the impact of anesthetic agents on retinal structure/function in developing animals. Several questions come to mind: In particular, what is the impact of longer periods of exposure? What are the effects of IV anesthetic agents? Does retinal cell death temporally reflect that occurring in the brain, and is such an index reliable? More specific to the findings reported in this study: Why are TUNEL and Annexin V binding after isoflurane exposure primarily found in the inner nuclear layer and not in the RGC layer? Of all retinal cells, the RGCs and their axonal projections most closely resemble those in the brain, and in fact, RGC axons project into the brain. Neither TUNEL nor caspases-3 and -8 expression nor Annexin binding with TUNEL exhibit temporal correlations in this study, which suggests that some immunolabels and/or methodologies may provide more accurate insights into retinal cell apoptosis than others.

A significant limitation of this study is that anesthetic agent-induced apoptosis has yet to be correlated with behavioral or cognitive impairment.[19] Unfortunately, the authors did not provide any behavioral or neurophysiologic data (e.g., electroretinography)[20] to support their findings. The relevance of this model to humans is not clear, because the postnatal day 7 in mice may be more analogous to a late gestation time point in humans.[21]

These limitations aside, the authors have certainly opened up a new area worthy of investigation, in which the possibility exists to examine the impact of anesthetic agents on brain development using the retina as a metric. If indeed retinal changes from anesthetic agents in the immature central nervous system mirror those in the brain, one day it may be possible to study such changes in real time using retinal imaging and thereby provide early warning of potential deleterious effects in individual patients.

DISCLOSURES

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