Good Gas, Bad Gas: Isoflurane, Carbon Monoxide, and Which Is Which?

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Modern pediatric medicine and surgery rely on highly skilled anesthesia management to achieve comfort and amnesia during painful and distressing procedures that are performed frequently in children of all ages. Over the last several decades, we have developed sophisticated anesthesia delivery and monitoring protocols that enable us to accurately assess and maintain stability of a child's physiological parameters, even under the most challenging surgical and pathological conditions or extremes of immaturity. The assured stability of cardiovascular and respiratory parameters led us to believe that an early exposure to anesthesia causes no harm to the child's development. However, over the last decade, we are being forced to reconsider our notion of anesthesia safety during critical periods of brain development. Numerous reports of harmful effects of anesthetics on neuronal and cognitive growth in young animals, and slowly emerging evidence in humans, suggest potentially harmful and long-lasting behavioral sequellae.

The very initial findings have suggested that clinically relevant anesthetics, alone or in combination, induce significant and widespread neuroapoptotic degeneration of developing neurons in immature rats.[1–3] Over the years, additional mammalian species (e.g., mice, guinea pigs, pigs, and nonhuman primates) were found to be susceptible to anesthesia-induced developmental neuroapoptosis.[4–8] Although the initial insult is very robust and ultimately leads to neuronal deletion,[9] signs of damage in the remaining neurons, though more subtle and hence often detected only at the ultramicroscopic or functional level, are observed some weeks after the initial insult and are impressive as well. Anesthetic effects on the remaining neurons are manifested as significant damage to synapse formation, stability and function,[3,10–13] impressive fragmentation of neuropil, and distortion of mitochondrial morphogenesis and regional distribution.[11,14,15] Hence, anesthesia-induced neurotoxicity is not a transient phenomenon but more of an ongoing process exhibiting different pathomorphological characteristics.

The main impetus for improving our understanding of anesthesia-induced neurotoxicity was fueled by very early findings, suggesting that the morphological impairments in young rodents are followed by impaired cognitive abilities.[3] Of particular concern was the fact that the gap in learning widened in adulthood and was manifested as inability to master more complicated learning paradigms.[3] Similar observations of delayed learning and diminished accuracy in the performed tasks were made in nonhuman primates exposed to anesthesia in early infancy.[16] Although a direct causal link between morphological impairments and cognitive delays has not yet been confirmed,[17,18] strategies aimed at curtailing neuronal damage have been effective in preventing or ameliorating anesthesia-induced cognitive impairments.[17]

Since the emerging retrospective clinical studies suggest a potential link between an early exposure to anesthesia and behavioral sequellae later in childhood,[19–22] there is urgency to improve our understanding of the mechanisms responsible for the neurotoxicity so that the most effective protective strategies can be introduced into clinical practice.

Isoflurane was recognized early on as one of the most neurotoxic volatile anesthetics not only in the severity of morphological damage but also in the seriousness of the behavioral impairments.[3,23] Pathomorphological markers suggest that isoflurane causes dose-dependent and widespread neuronal death that is apoptotic in nature and easily detected by monitoring caspase-3 activation, the final step leading to DNA fragmentation and the formation of apoptotic bodies.[3] Although both intrinsic and extrinsic pathways of apoptosis play an important role in caspase-3 activation,[24] activation of apoptosis by isoflurane is primarily via the intrinsic pathway, that is, it is mitochondria-dependent. Isoflurane damages mitochondrial integrity and impairs the function of scavenging enzymes.[14] This in turn causes overproduction of superoxide ions and hydrogen peroxide (a byproduct of superoxide dismutation), resulting in oxygen-free radical overload that ultimately leads to excessive lipid peroxidation of mitochondrial inner and outer membranes.[17] These actions have been linked to further compromise in mitochondrial integrity[11,15,17] and cytochrome c leak.[24] Cytochrome c, in turn, activates caspases-9 and -3 and causes a cascade of events ultimately leading

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to DNA fragmentation, formation of apoptotic bodies, and neuronal demise.[24] Although the downstream mechanisms have been well worked out, the initial step that promotes excessive cytochrome c leak is still under investigation.

In this issue of the journal, Drs. Cheng and Levy[25] suggest that the initial step may involve isoflurane-induced upregulation of cytochrome c peroxidase activity. They suggest that in the presence of hydrogen peroxide, cytochrome c oxidizes cardiolipin to hydroperoxycardiolipin, that mobilizes cytochrome c from the inner mitochondrial membrane and enables it to be released after permeabilization of the outer membrane, leading to a vicious cycle of further cytochrome c leak, upregulated oxygen-free radical production, lipid peroxidation, and protein oxidation. With the addition of this insight, we have a better understanding of the cascade of events initiated in mitochondria and resulting in neuroapoptotic degeneration (Fig. 1).

The major advance provided by improved understanding of anesthetic-induced neurodegeneration is the potential to design clinically attainable methods to protect against anesthesia-induced developmental neurotoxicity. A review of all neuroprotective strategies is not within the scope of this editorial, but it is noteworthy that some neuroprotective approaches focus on curtailing excessive oxygen-free radical production, lipid peroxidation, and on protecting mitochondrial function and integrity, thus minimizing cytochrome c leak.[17,26] In their study, Drs. Cheng and Levy[25] bring to our attention another potential clinically relevant method, which relies on coadministration of subclinical concentrations of carbon monoxide (CO) (resulting in the carboxyhemoglobin [COHb] levels lower than the ones known to be symptomatic in humans, around 10% and higher). They report that administration of 5 ppm CO for only an hour during isoflurane (at 2%) anesthesia in 7-day-old mouse pups (around the peak of mouse brain vulnerability) results in significant decrease in caspase-3 activation in neurons of the somatosensory neocortex, hippocampus, and hypothalamic/thalamic regions. This concentration of inhaled CO does not cause significant increase in COHb level compared with inhaled air (sham controls). When a significantly higher inhaled CO concentration (100 ppm) was coadministered, they noted an additional decrease in isoflurane-induced neuroapoptosis but at the risk of generating 3- to 4-fold higher blood levels of COHb (3%–4%) compared with sham controls. Nevertheless, the authors claim that both low (5 ppm) and higher (100 ppm) concentrations of CO should be considered subclinical and not harmful.

A closer look at potential mechanisms for CO-induced neuroprotection suggested that isoflurane-induced cytochrome c peroxidase activity and cytochrome c leakage were ameliorated by CO coadministration. However, the authors do not confirm that isoflurane causes upregulation of oxidized cardiolipin or that CO prevents excessive cardiolipin oxidation, an important step in compromising mitochondrial integrity. It is important to note that they do not examine whether CO coadministartion protects against isoflurane-induced cognitive impairments, a demonstration that would be necessary to establish potential clinical

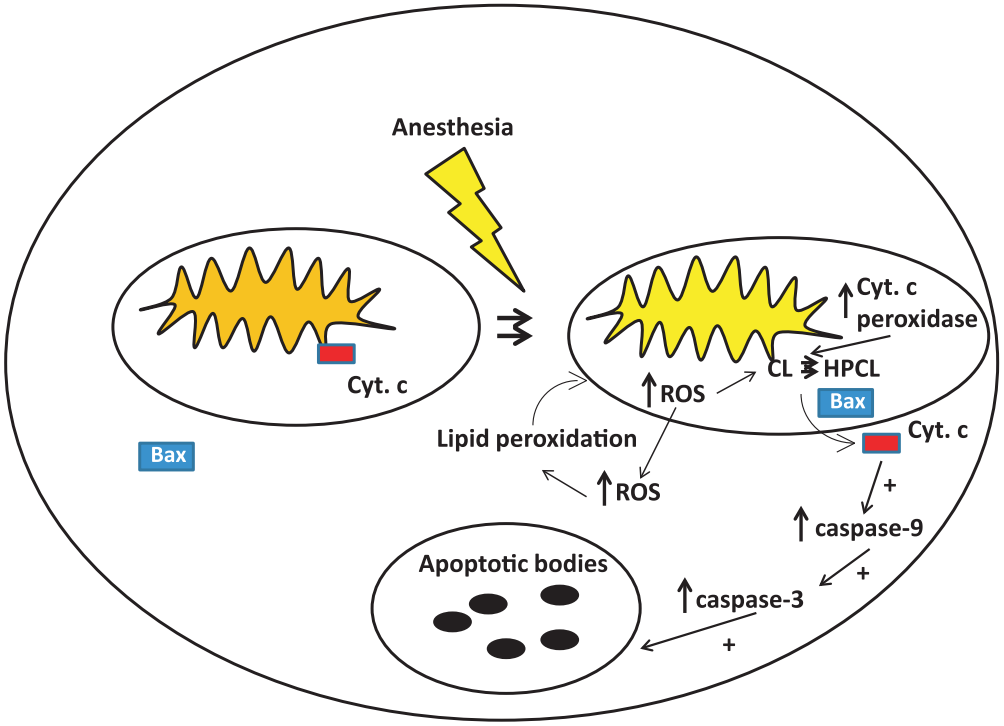

Figure 1. Proposed schematic diagram of mitochondria-dependent, isoflurane-induced apoptotic pathways. Isoflurane promotes translocation of Bax from cytosol to the mitochondrial membrane. In addition, isoflurane increases the activity of cytochrome c (Cyt. c) peroxidase, which results in increased conversion of cardiolipin (CL) to hydroperoxycardiolipin (HPCL). Both events cause an increase in mitochondrial permeability. This, in turn, mobilizes cytochrome c from the inner mitochondrial membrane, enabling it to be released after permeabilization of the outer membrane and leading to a vicious cycle of reactive oxygen species (ROS) upregulation, lipid peroxidation, and compromised mitochondrial integrity and further cytochrome c leak. Released cytochrome c activates caspase-9 and then caspase-3, leading to DNA fragmentation and the formation of apoptotic bodies.

relevance. The lack of cognitive correlates, especially, is of concern since the latest publication by Drs. Cheng and Levy and their colleagues suggests that CO exposure alone (at 5 or 100 ppm) for 3 hours caused apoptotic neurodegeneration in young mice, followed by significant cognitive deficits and impairment in social interactions.[27]

In summary, this study addresses an important issue in developmental neurobiology and moves us a step closer to understanding the pathways responsible for anesthesia-induced neuroapoptosis. Although the use of CO may seem extreme based on devastating outcomes reported with CO asphyxia, CO is endogenously produced, and during low-flow general anesthesia, it is known to result in a slight increase in COHb (<1%). In that sense, the 5 ppm concentration used in this study that resulted in similar levels of COHb could be considered "physiological," thus suggesting a potentially useful and readily available neuroprotective tool. A remaining problem is that the amelioration of apoptotic activation, though significant, was not complete, thus leaving many immature neurons vulnerable and unprotected even with the higher inhaled CO concentration (100 ppm).

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