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Isoflurane Neuroprotection

A Passing Fantasy, Again?

DATING from the first report that isoflurane produces profound reduction in cerebral metabolic rate at clinically relevant concentrations,¹ it has been postulated that isoflurane can provide perioperative neuroprotection. If so, this would be a major advance over using barbiturates for the same purpose. To achieve similar cerebral metabolic rate reduction with barbiturates, patients are committed to hours of postoperative coma, hardly an ideal circumstance for monitoring neurologic status. Considerable work has been dedicated to examining the neuroprotective properties of isoflurane. In this issue of ANESTHESIOLOGY, Kawaguchi *et al.*² present new information important to the debate regarding isoflurane neuroprotection. What they have shown is that isoflurane indeed provides neuroprotection against a focal ischemic insult. However, at least in the case of their laboratory model, the protection provided is not permanent. Over time, the tissue that was initially protected by isoflurane went on to die, and the final result was no different than that found in rats subjected to the ischemic insult in the absence of isoflurane.

A historical perspective is useful in placing this observation in context. Early investigations provided evidence that isoflurane is neuroprotective. Mice subjected to hypoxia survived longer in the presence of isoflurane, and isoflurane-anesthetized dogs exposed to hemorrhagic shock had better preservation of brain adenosine triphosphate concentrations.³ Rats subjected to hemi-

spheric global ischemia had better ischemic outcome when given isoflurane anesthesia *versus* nitrous oxide sedation.⁴ In humans undergoing carotid endarterectomy, the critical cerebral blood flow threshold required for electroencephalographic changes indicative of ischemia was substantially lower during isoflurane *versus* halothane anesthesia.⁵

However, none of the aforementioned studies was perfect in experimental design, and methodologic criticism of those investigations was buoyed by other reports that isoflurane offered little benefit to ischemic brain tissue. When baboons were anesthetized with isoflurane, thiopental, or fentanyl-nitrous oxide during transient middle cerebral artery occlusion (MCAO), the isoflurane group had worse outcome than the thiopental group.⁶ In fact, it was nearly statistically significant ($P = 0.07$) that the isoflurane group had a worse outcome than the fentanyl-nitrous oxide group. In a study of monkeys subjected to MCAO combined with induced hypotension (so as to model cerebral aneurysm surgery), there was no difference in outcome between isoflurane- and halothane-anesthetized animals.⁷ Because halothane was thought to have no neuroprotective potential, it could be concluded that isoflurane also was devoid of this property. Finally, in rats subjected to severe forebrain ischemia, there was no advantage of isoflurane anesthesia *versus* nitrous oxide sedation.⁸ In addition to dashing hopes that we at last had a safe, convenient modality for intraoperative neuroprotection, these studies were used to make the case that cerebral metabolic rate reduction is an insufficient criterion for predicting neuroprotection by pharmacologic agents.⁹ Accordingly, the burden of proof was returned to proponents of isoflurane neuroprotection. Methodologically sound evidence was required to prove this benefit.

Although it took several years for that evidence to emerge, research teams eventually took advantage of advances in laboratory models of ischemic brain injury to re-address this issue. One problem constantly inhibiting clear definition of neuroprotection by anesthetics was the necessity to anesthetize both control animals as well as those receiving the anesthetic under investiga-

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tion. The control anesthetic could only be presumed inert. If both the control and experimental groups had equivalent outcome, it remained plausible that both anesthetics actually provided the same (and perhaps substantial) neuroprotection.

Modeling of ischemic insults was enhanced by introduction of the rat filament MCAO technique in which a nylon monofilament is advanced into the circle of Willis *via* the carotid artery. This relatively noninvasive procedure allowed the filament to be positioned during anesthesia, but the rats could then be rapidly awakened and left fully awake for the major portion of the occlusive insult. Under these conditions, halothane anesthesia continued throughout the insult was found to cause profound reduction in cerebral infarct size relative to that occurring in animals allowed to awaken.¹⁰ This finding was soon extended to the use of isoflurane. Although rats anesthetized with fentanyl-nitrous oxide had infarct sizes similar to those allowed to awaken, infarct size was nearly halved in those given isoflurane.¹¹ It was then asked if isoflurane was protective against global ischemia, an insult that is famously resistant to neuroprotection by pharmacologic agents. Indeed, rats had major reduction in damage to selectively vulnerable cerebral structures when anesthetized with isoflurane *versus* fentanyl-nitrous oxide.¹² Lowly isoflurane? The magnitude of the aforementioned neuroprotective effects is similar to that of the finest compounds currently under development by pharmaceutical companies as neuroprotective agents. Given that these studies used modern methods to assure absence of confounds from nonspecific anesthetic effects on brain physiology, it is logical to accept this data as definitive evidence that isoflurane is effective in inhibiting necrotic processes resulting from cerebral ischemic insults.

If isoflurane is neuroprotective, how does it work? One hypothesis is that isoflurane inhibits excitotoxicity associated with accumulation of glutamate in the extracellular space during ischemia. Indeed, isoflurane has been shown *in vitro* to be an antagonist of glutamate receptors, thereby diminishing deleterious calcium influx.^{13,14} Isoflurane is also a γ -aminobutyric acid (GABA_A) receptor agonist.¹⁵ It has been shown numerous times that up-regulation of GABAergic activity can serve to diminish glutamate excitotoxicity.^{16,17} Finally, isoflurane has been shown to reduce release (accumulation) of glutamate in the extracellular space during ischemia.¹⁸ All of these properties of isoflurane can be presumed to contribute to reduced necrotic cell death.

One issue remained unexplored. Du *et al.*¹⁹ provided

evidence that not only is necrosis a sequel to ischemia, but so, too, is apoptosis. Rats were subjected to 30 or 90 min of MCAO. After 1-day recovery, infarcts were absent in the 30-min group but large in the 90-min group. In contrast, when the rats were examined 2 weeks after ischemia, infarcts were large in both the 30- and 90-min groups. There is sufficient evidence in the literature to presume that, although necrotic processes were not initiated by the 30-min insult, processes were initiated that invoke inborn properties of cells to commit suicide (*i.e.*, apoptosis).²⁰ However, a greater postischemic interval is required for apoptotic lesions to mature. Indeed, none of the recent trials demonstrating isoflurane neuroprotection had examined recovery intervals of greater than 1 week.

This is why the work of Kawaguchi *et al.*² is so important. Rats subjected to filament MCAO while awake or anesthetized with isoflurane were allowed survive 2 days or 2 weeks. The isoflurane group had major protection at 2 days. At 2 weeks, however, both the awake and isoflurane groups had large and similar-sized infarcts. Because this work meets all current methodologic standards, it must be taken as a warning of potential limitations of isoflurane neuroprotection. Further work is required to confirm that apoptosis was initiated and that there was no inhibition of these events by isoflurane. Before we can confidently state that isoflurane neuroprotection is only transient, this work must also be confirmed by other laboratories. This is particularly true because the coefficient of variation was large in both 2-week recovery groups. Therefore, the statistical power to detect an effect of isoflurane was low. Finally, we do not know if this transient effect is specific to focal ischemia or is also true for global ischemia.

As a result of the work by Kawaguchi *et al.*,² we are again in an era of uncertainty regarding isoflurane neuroprotection. Conclusions drawn from laboratory models of ischemic brain injury are often criticized because they lack sufficient rigor in testing before results are extrapolated to human conditions. We must continue to examine interactions between anesthetics and ischemic brain because anesthetics are used frequently in patients at risk for such insults. Kawaguchi *et al.*² have made an important contribution to this process.

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