

Anesthetic Effects on the Developing Nervous System

If You Aren't Concerned, You Haven't Been Paying Attention

ANESTHESIOLOGISTS have long been concerned with the possibility that anesthetic and analgesic agents administered during pregnancy could have deleterious effects on the fetus. More recently, this concern has escalated and extended to include the period of neonatal and early childhood development. Although experimental support for this concern existed decades ago,¹ the current controversy can be traced to the seminal observation that administration of *N*-methyl-D-aspartate glutamate antagonists, including the anesthetic ketamine, could trigger widespread apoptotic neurodegeneration in the developing brain.² Further experiments demonstrated that a similar response could be induced by γ -aminobutyric acid agonists.³ Taken together, these findings have implicated a broad and diverse group of compounds, including nearly every commonly used anesthetic and sedative-hypnotic. Although some studies have been able to correlate these effects with behavioral changes in animals,⁴ the clinical relevance remains obscure. Similarly, there are observational clinical studies that have generated correlative evidence linking early anesthetic exposure with developmental impairment,^{5,6} but these are potentially contaminated by confounding variables, such as concurrent comorbidities, surgical procedures *per se*, perioperative events, and possible links between the underlying condition requiring surgery and early neurodevelopment. Not surprisingly, the uncertain clinical significance of these experimental findings and clinical observations have ignited a firestorm of controversy, which has generated as much heat as illumination, evidenced by numerous editorials, review articles, and letters to the editor, and the extension of this discussion to the mainstream media. In response, the Food and Drug Administration convened an Advisory Committee Meeting in April 2007.* Among their conclusions was the need for studies evaluating the vulnerability of the immature spinal cord to neuraxial anesthetics and analgesics, the import of which is reinforced by data suggesting that even exposure to general anesthesia can provoke apoptosis in the spinal cord of neonatal rat.⁷ Accordingly, in this issue of ANESTHESIOLOGY, two articles from an international collaborative research group de-

scribe the development of a neonatal spinal toxicity model and its application to the assessment of the toxicity of morphine and ketamine.^{8,9}

The first of the two articles describes the establishment of the intrathecal injection technique that is performed on neonatal rats at postnatal (P) days 3, 7, and 21 (P3, P7, and P21, respectively), which is validated by both *in vivo* and postmortem evaluation of injectate distribution.⁹ These developmental ages roughly span the period of “rapid brain growth spurt” and accelerated synaptogenesis in rats, which extends from around birth until 2 weeks of age.¹⁰ The equivalent vulnerable period in humans is more protracted, starting around the third trimester and persisting for perhaps 2 or 3 postnatal years,¹⁰ although this is a point of considerable debate.¹¹ After the assessment of baseline mechanical withdrawal responses, escalating doses of morphine were administered to determine the antinociceptive threshold and maximum tolerated dose. Sections of the spinal cord and proximal nerve roots were subjected to histopathologic examination, including the assessment of activated caspase-3 immunoreactivity and Fluoro-Jade C staining, as markers for apoptosis and neuronal degeneration; long-term functional assessment was made using sensory thresholds and gait analysis at 5 weeks of age.

The safety margin for morphine was found to be extremely favorable. Even at the highest tolerated doses, which were limited by side effects (*e.g.*, respiratory depression), morphine failed to induce histologic damage, evidence of enhanced neuroapoptosis or long-term sensory or gait disturbance. Degenerating neurons and activated caspase-3 cells were present 1 day after injection in P3 animals, largely restricted to the dorsal horn. This seemed to reflect normal developmental apoptosis, as the number of such cells was significantly lower in P3 animals examined 7 days postinjection, rarely found in P21 animals, and were similar in those receiving morphine or saline, and in age-matched naïve animals. Being limited by side effects, determination of the actual thera-

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◆ This Editorial View accompanies the following articles: Walker SM, Westin BD, Deumens R, Grafe M, Yaksh TL: Effects of intrathecal ketamine in the neonatal rat: Evaluation of apoptosis and long-term functional outcome. ANESTHESIOLOGY 2010; 113:147–59; and Westin BD, Walker SM, Deumens R, Grafe M, Yaksh TL: Validation of a preclinical spinal safety model: Effects of intrathecal morphine in the neonatal rat. ANESTHESIOLOGY 2010; 113:183–99.

peutic index (toxic dose/antinociceptive dose) for neurodegeneration could not be determined, but they were at least 300 at P3 and 20 at P21. (It should be apparent that the higher safety margin for the younger animals was not a reflection of resistance to toxicity, but rather their greater sensitivity to morphine-induced antinociception, effectively reducing the denominator of the equation.)

Although intuitively attractive, an underlying assumption in the use of the therapeutic index for the assessment of clinical safety is that the desired effect and toxicity are mediated by the same mechanism or at least run parallel. This may not be the case,¹² particularly across species and developmental age. An alternative method routinely used in drug development is to determine the highest dose that does not produce any discernable toxicity, that is, the no observed adverse effect level (NOAEL), to estimate a safe human equivalent dose (HED). However, such estimates are hindered by imprecise knowledge regarding equivalence, and some have argued that the therapeutic index has greater validity.¹³ Most critically, the application of such alternative analysis to the current findings does little to change the apparent neurotoxic safety of intrathecal morphine. Specifically, with systemic administration, it is generally assumed that the dose roughly tracks with body surface area but with discrete anatomic compartments, such as the subarachnoid space, it is more common to base estimates of equivalence on the relative size of these compartments in the experimental animal compared with the human. Applying the most conservative estimates to the current data, the human equivalent dose would be at least 2.5 mg/kg for intrathecal administration, providing roughly a 250-fold safety margin. Moreover, this is the minimal safety factor with respect to neurodegeneration, as toxicity testing was limited by side effects.

The second article explores the intrathecal toxicity of ketamine, an anesthetic that has been the most extensively studied and the most commonly implicated in developmental neurotoxicity.⁸ The lowest dose that reversed inflammatory hyperalgesia in the P3 animals was 3 mg/kg, and this was associated with accelerated neuroapoptosis within the dorsal horn, and long-term effects on mechanical withdrawal (hyperalgesia) and gait disturbance. This proapoptotic effect of ketamine was development dependent, because there was only a nonsignificant increase in the number of degenerating neurons and activated caspase-3 cells with injections made at P7 and no apparent trend with P21 injections. Thus, as expected, vulnerability to ketamine coincided with the period of normal developmental apoptosis. The studies examining the effect of systemic anesthetics on apoptosis in the developing brain have generally found a peak effect around P7,¹⁴ and the earlier period of vulnerability in the current experiments likely reflect the relatively advanced maturation of the spinal cord. This may, in part, explain the failure to find any functional impairment in the aforementioned studies demonstrating spinal neuroapoptosis induced by general anesthesia, as animals were exposed to anesthetic at P7.⁷ A second distinction is the greater effect on the ventral horn observed in these previous studies, which is a bit surprising given the relatively

early development of this region of the cord.¹⁵ Further studies investigating the effects of systemic anesthetics on the spinal cord neuroapoptosis and functional impairment are certainly needed and should include *in utero* exposure.

The results with ketamine are clearly a stark contrast to those obtained with morphine. Because toxicity occurs at a dose at or below that required for effect, the therapeutic index is unity or inverted. If toxicity is assessed using conversion of the no observed adverse effect level to the human equivalent dose with parameters similar to those applied to morphine, the safety margin relative to the dose commonly administered for pediatric caudals is less than 2. Although there is a greater potential for toxicity with intrathecal injection as opposed to epidural injection, inadvertent intrathecal administration can, and does, occur in clinical practice. Further, even if the greater tolerance to epidurally administered drugs is considered, the safety margin would likely remain less than 10, a commonly used standard for the maximum recommended starting dose for human clinical trials. Finally, in contrast to morphine, the no observed adverse effect level is no greater than the lowest dose studied, rather than no lower than the highest dose. Although additional studies could more precisely define the no observed adverse effect level for ketamine, they are unnecessary—even in adults, the safety of spinal ketamine is questionable, and given its marginal therapeutic benefit, it would be difficult to mount a coherent argument for its continued use as an adjuvant for pediatric caudal anesthesia.

These two articles thus describe the development and validation of a model that has potential utility for investigations of the effect of intrathecal anesthetics or analgesics on early postnatal development. The impressive therapeutic index demonstrated with morphine combined with the apparent toxicity of ketamine serves to establish the model's sensitivity and specificity, providing some degree of confidence in the model's validity. What is not included in these systematic investigations is an assessment of the local anesthetics, which are obviously the mainstay of pediatric regional anesthesia. Despite the apparent safety of the local anesthetics, there may be reason for concern. In cell culture, lidocaine and other local anesthetics are capable of inducing apoptosis, and the concentrations at which these effects occur are lower than those associated with necrotic cell death.¹⁶ Second, experiments conducted in our laboratory on adult rats demonstrate significant histopathologic effects, including axonal degeneration or demyelination, in the nerve roots at intrathecal doses below that required for reliable "surgical" anesthesia. These histologic effects are "subclinical" in our model, that is, they do not produce obvious motor dysfunction or persistent changes in tail-flick latency. Whether similar effects occur in the neonate, and to what degree, is of obvious importance and amenable to investigation in this newly developed *in vivo* model. Further, such preclinical data have utility beyond demonstration of the expected safety of these techniques in this potentially vulnerable population. As commented by the Food and Drug Administration Advisory Committee,* the most convincing evidence for safety or toxicity of the general

anesthetics would be derived from randomized controlled trials. Given the confines of ethical clinical research, randomization to neuraxial *versus* general anesthesia is certainly among the most, if not the most, logical design, and such studies are just getting underway.¹⁷ Moreover, not to add confusion to the discussion, there is some evidence to suggest that pain *per se* can induce neurodegeneration that can be blunted by anesthetics.¹⁸ Neuraxial techniques may be the most effective strategy to prevent or attenuate such effects.

It should be mentioned that the literature in this field is not entirely consistent.¹⁹ Nonetheless, there is adequate evidence to support concern. Moreover, recent experimental studies have linked anesthetic exposure to changes in dendritic spine architecture.²⁰ This raises the question of whether anesthetics may interfere with neural network formation beyond the period of neuroapoptotic vulnerability. The current state of affairs can perhaps be best summed up by a comment made by Charles DiMaggio, coauthor of one of the relevant epidemiologic studies.⁵ When asked in an interview about the clinical significance of the study's findings, he responded, "The jury is still out; actually, the jury hasn't even retired to deliberate."[†]

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