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Sublethal spinal ketamine produces neuronal apoptosis in rat pups

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may have received less narcotic than their younger counterparts—or that these patients take drugs that may play a role in cancer recurrence, such as β blockers and statins.⁴

Mohamed Tiouririne, M.D., University of Virginia, Charlottesville, Virginia. mt9y@virginia.edu

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In Reply:

We thank our colleagues for their interest regarding our recent work.¹ In response to their inquiries, *recurrence* in our study was defined as any (local or metastatic) detection of colon cancer after primary resection. In the commonwealth of Virginia, treating physicians are required by law to report the cancer status of all patients. The University of Virginia Cancer Center, Charlottesville, tracks this data. Therefore, we are fortunate to have access to long-term follow-up cancer recurrence data on a large number of patients. However, we fully acknowledge that any retrospective study, including ours, is limited by (1) the accuracy of the available medical records, which may include missing data, and (2) difficulty controlling bias and confounding factors that could influence cancer recurrence (*e.g.*, α and β blockers, statins, nonsteroidal antiinflammatory drugs, cyclooxygenase inhibitors).

We agree with Dr. Tiouririne that intraoperative use of epidural analgesia (*i.e.*, to supplement general anesthetics) may have different effects on cancer recurrence than epidural analgesia used only postoperatively. As Christopherson *et al.*² note, a variety of factors influence cancer recurrence. For example, cancer stage and grade are almost always the best predictors of recurrence. Although our analysis corrected for major factors, our statistical modeling was, of course, restricted to the available data.

Both letters assert that our findings contradict those of Christopherson *et al.*² However, this interpretation of our results is inaccurate; neither we nor Christopherson *et al.*² found an overall (primary hypothesis) benefit of epidural analgesia. Unplanned *post hoc* subgroup analyses—including

our observation that cancer recurrence was reduced in older patients who received epidural analgesia—are notoriously unreliable. Indeed, such analyses, when statistically significant at 0.05, have only a 57% chance of being replicated in an identical clinical trial.³

Although the idea that regional analgesia may reduce the incidence of cancer recurrence is exciting, it remains a hypothesis at this time—a question that can be answered only with prospective randomized clinical trials. Fortunately, several such studies are already in progress.

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Sublethal Spinal Ketamine Produces Neuronal Apoptosis in Rat Pups

To the Editor:

Sir, we read with interest the article by Walker *et al.* and the accompanying editorial view.^{1,2} Undoubtedly, subarachnoid administration of large doses of ketamine produces neuronal apoptosis in newborn rats, as was eloquently demonstrated by this article. However, we would like to request further clarification regarding the statement “3 and 10 mg/kg produced increasing initial sedation, and higher doses were lethal.” Unlike the corresponding article regarding the safety of intrathecal morphine in rat pups in the same issue,³ no indication of calculated LD₅₀ of intrathecal ketamine is given. We are not suggesting that similar dose response curves need to be constructed^{4,5} but would welcome the publication of supporting data.

Rat pups were also exposed to smaller doses of intrathecal ketamine (0.1–0.3 mg/kg); again, no data on analgesic action or neuronal apoptosis are given. These doses (rather than more than 3 mg/kg) are the comparative and relevant equivalents commonly employed for caudal anesthesia.⁶

We have also some concerns regarding reporting of the apoptosis data.¹ First, the authors are assuming that the cells they are staining with active caspase-3 are indeed neurons without assessing the cell type. Second, the authors have

failed to fully normalize the data in that they have just counted the number of positive cells, be they Fluoro-Jade C or caspase-3 positive, in each field of view. This method does not take into account the number of cells present in the section or the size of the section. One method is based on the generation of a “wandering mean.” To generate these data, the following procedure should be undertaken. Count the number of events (caspase-3 positive or Fluoro-Jade C positive cells) and the total number of relevant cells in the first microscopic field. This will give the first apoptosis score (A1 based on N1 cells). In the second field, the process is repeated and running scores recorded to give a running mean (A2 based on N2 cells). This process is repeated to give multiple running averages (A3, N3 . . . An, Nn). If these are plotted, the mean will be seen to wander and eventually oscillate about a mean value, and as N increases, this will become less. This procedure can then define experimentally the number of events to be assessed to produce a given quality of data.⁷

Intrathecal ketamine may have a much narrower intrathecal therapeutic index compared with that of morphine. However, local anesthetic agents have been shown to have detrimental effects on neuronal apoptosis,⁸ and the right balance between exposing vulnerable children to potential harmful general or regional anesthetics is yet to be established. Until then, we need to pay attention to the primary cause of morbidity and mortality in children: hypoxia.⁹

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In Reply:

We thank Drs. Engelhardt, Blaylock, and Weiss for their comments on our article,¹ and we are happy to clarify the issues of ketamine dosing. Intrathecal ketamine (30 mg/kg) produced irreversible sedation and respiratory depression in P3 pups, and excitation and convulsions in P21 animals after emergence from anesthesia that necessitated termination. As a result, the same degree of dose escalation was not possible with ketamine as with morphine. Rather than indicating that “sublethal doses” of ketamine are associated with apoptosis, this emphasizes the narrower therapeutic window between analgesia and dose-limiting side effects with ketamine.

The authors incorrectly stated that “no data on analgesic action” were provided for 0.1–0.3 mg/kg intrathecal ketamine. Figure 1B clearly presents dose-response data for antihyperalgesic effects of intrathecal ketamine in both P3 and P21 pups.¹ Because of ketamine's mode of action, increases in baseline sensory threshold (*i.e.*, antinociceptive effects) are not seen. The increased primary afferent input after injury (*i.e.*, carrageenan-induced hind paw inflammation) results in activation of *N*-methyl D-aspartate-mediated sensitization in the spinal cord, and dose-dependent reversal of hyperalgesia by ketamine can now be demonstrated. This pattern of response is discussed and referenced under “Dose-dependent effects” in our manuscript. Significant reversal of hyperalgesia was seen 30 min after intrathecal ketamine 3 mg/kg in P3 rats and 15 mg/kg in P21 rats. As we had shown that ketamine produced apoptosis within this analgesic dose range in P3 pups, repeating the same experiments with subtherapeutic doses would represent unnecessary use of animals and resources.

Engelhardt *et al.* refer to doses that “are the comparative and relevant equivalents commonly employed for caudal anesthesia.” We are surprised that the authors expect there to be a direct correlation between the doses used in different species, at different ages, and by different routes. Again, these issues were covered in our discussion.¹ Our rationale for describing results in terms of a therapeutic index was to provide a ratio of toxic to functional doses that could facilitate comparison of different drugs at different developmental

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The above Letter was sent to the author of the associated Editorial View, who declined to respond to the Letter.—James C. Eisenach, M.D., Editor-in-Chief.