Letter to the Editor

Dear Editors,

We read with great interest the recent article by Laferriere and Pang entitled, "Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents".¹ The authors made a statement about technique in intraperitoneal injections that we wish to comment on. They make the point that "Before injection, it is often suggested to aspirate the needle to assure its correct placement in the peritoneal cavity, although there is no evidence supporting the usefulness of this practice." (p 255). Intraperitoneal (IP) injection is commonly referred to as the "blind stick" technique. This route of administration is usually preferred for injection of larger volume doses with less risk of drug escaping into other compartments as may happen with intravenous or other injections. Needle aspiration is used routinely to confirm intravenous placement and serves both to confirm the location of the needle tip inside a vein, but also to keep the investigator from developing the bad habit of assuming that injection anywhere into the abdomen will constitute a satisfactory IP delivery of drug. We teach all persons as part of our hands-on training for animal experimentation to aspirate as part of the IP injection technique. We stress an approach into the right lower abdomen and insist that investigators demonstrate that they aspirate only air or occasionally clear intraperitoneal fluid before they deliver their intended drug dose.

As a veterinary technician with 17 years in research and the principal trainer for rodent handling over the past 5 years, I have seen the tendencies of students and more senior investigators to select oversized needles and be careless in their technique. While there may not be published studies to demonstrate the utility of aspiration, I strongly believe that the extra step is important for keeping investigators focused on proper technique that will minimize unintended consequences of damaging the bowel, causing intraabdominal bleeding, or delivery of drug into the intestinal lumen. Best Regards,

Stacy Stephenson. AAS, RLATg. Instructional Support Technician SUNY Downstate Health Sciences University 450 Clarkson Avenue, Brooklyn, NY 11203 Division of Comparative Medicine BSB 8-62, Box 47, P: 718-270-4198 O: 718-270-1194

Reference

 Laferriere CA, Pang DS, 2020. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. J Am Assoc Lab Anim Sci 59:254–263. https://doi. org/10.30802/AALAS-JAALAS-19-000081.

Response to Stacy Stephenson Letter to the Editor: Dear Drs Compton and Toth,

Thank you for the opportunity to respond to this letter commenting on our recent review.² We thank Ms Stephenson for her comments and interest in our work.

We agree that aspirating before injecting is important, and standard practice when performing intravenous injections. We do not dispute that aspirating before injecting, in general, constitutes good practice (along with appropriate restraint and equipment selection). The sentence quoted from our review reflects this, in addition to stating that, to the best of our knowledge, there is no evidence supporting the benefit of aspirating before IP injection. Good injection technique encompasses multiple factors, not limited to aspirating before injection. In the section of the review, "Intraperitoneal injection technique", our intention was to describe those factors for which there is supporting evidence (such as restraint technique, body position, injection site). By drawing attention to the absence of evidence supporting aspiration before IP injection, it was not our intention that this be interpreted as a call to stop this practice. We apologize if this is the impression given. In our own practice we habitually aspirate before IP injection, though this largely reflects a well-established habit rather than a belief that it is a sensitive or specific method to identify a misplaced needle tip.

To confirm or refute the value of aspirating before IP injections, research is needed, preferably reporting differences in outcome with different gauges of needle. Later in our review, we stated that "cecal and intestinal content may not easily be aspirated through a small-gauge needle". This was based on a statement made in the discussion section of Gaines Das and North (2007).^{1,a} Until such evidence is provided, the practice of aspirating is unlikely to be harmful (beyond the small amount of time added to the act of injection), but its benefits are unclear. For example, if clear fluid is aspirated (the example given by Ms Stephenson), it is unclear to what extent this confirms needle location outside an organ such as the bladder. To establish this association, the results of aspiration would need to be compared with findings at necropsy.

Yours sincerely,

Daniel Pang and Colin Laferriere

Reference

- 1. Gaines Das R, North D. 2007. Implications of experimental technique for analysis and interpretation of data from animal experiments: outliers and increased variability resulting from failure of intraperitoneal injection procedures. Lab Anim 41:312–320. https://doi.org/10.1258/002367707781282802.
- Laferriere CA, Pang DS, 2020. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. J Am Assoc Lab Anim Sci 59:254–263. https://doi. org/10.30802/AALAS-JAALAS-19-000081.

^aNote: In preparing this letter, we realized that this statement was misattributed to reference 4 of the review instead of the correct reference, 40.

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