IACUC Policy on Retroorbital Bleeding and Injections in Mice and Rats

Purpose

To comply with government regulations (PHS and USDA), AAALAC, *The Guide for the Care and Use of Laboratory Animals,* and standard veterinary care techniques, The University of Rhode Island IACUC has implemented a policy concerning retro-orbital eye bleeding and injections in mice. Retro-orbital injection is an acceptable alternative to tail vein injection for the intravenous delivery of compounds, provided the individual is properly trained. While retro-orbital injections may seem aesthetically distasteful, when mastered, this technique is ultimately more humane than alternative intravascular injection techniques in mice and rats.

Training

1. In the hands of an unskilled phlebotomist, retro-orbital sampling has a greater potential than other blood collection routes to result in complications. When personnel are undergoing training in retro-orbital blood collections, general anesthesia for the animals is required, and the animals are euthanized immediately following the procedure.
2. Authorization for an investigator to perform retro-orbital bleeding will be granted by the IACUC only after the Attending Veterinarian (AV) or Certified Veterinary Technician (CVT) trainer has certified that an individual has become proficient at the procedure.
3. Protocol participants transferring from another institution may provide proof of training from that institution to the AV or CVT. The protocol participant will be required to display competency in the method to the AV or CVT

Requirements

Investigators should maintain familiarity with the IACUC guidelines regarding retro-orbital *sinus* bleeding in mice. In rats, the presence of a *venous plexus* rather than a sinus can lead to greater orbital tissue damage than in the mouse. Retro-orbital bleeding represents more than “minimal or transient pain and distress” and, according to the USDA, is an example of a “Category D” procedure.

1. All animals must be appropriately anesthetized prior to performing procedure. Since this is a short procedure, Isoflurane is recommended unless this procedure is to be followed by another, more lengthy procedure requiring the administration of injectable anesthetic agents. Place a funnel-shaped nose cone connected to a non-rebreathing apparatus on the anesthetized animal. Use a snorkel, other active waste gas scavenger, or a charcoal scavenging device to manage waste anesthetic gases and decrease exposure of personnel to the gases. In addition, a

topical ophthalmic anesthetic, e.g., proparacaine or tetracaine drops, is recommended prior to the procedure, and may be considered an analgesic.

2. For bleeding, sterile capillary tubes and pipettes should be used to help avoid periorbital infection and potential long-term damage to the eye. The edges of the tubes should be checked for smoothness to also decrease likeliness of eye damage.

3. For injections, use a 27-29 gauge, 0.5-inch, insulin needle and syringe (30-unit, U-100 would work best). A tuberculin syringe with a 27-29 gauge, 0.5-in hypodermic needle can also be used. When using a tuberculin syringe, a large volume (50–70 µl) of the injectate is lost in the dead space of the syringe hub and needle, which is important to keep in mind if the injectate is valuable. We recommend that injectate volumes not exceed 150 µl.

4. An individual can receive no more than one injection per day. When more than one injection is required, alternate between eyes, and allow at least 24 hours between injections. Do not exceed two injections per eye in any individual.

5. Maximum blood collection volumes (mouse):

- Weekly sampling (0.6% body weight): 115µL (25g mouse)
- Biweekly sampling (0.8% body weight): 200µL (25g mouse)
- Monthly sampling (no fluid replacement, 0.6%): 200µL (25g mouse)
- Monthly sampling (with fluid replacement, 1.5%): 350µL (25g mouse)

6. Maximum blood collection volumes (rat):

- Biweekly sampling (1.5% body weight): 3.5 mL (250g rat)

7. Volumes greater than the maximum volumes listed above require scientific justification and prior approval by the IACUC.

8. A minimum of 10 days should be allowed for tissue repair before repeat bleeding from the same orbit, otherwise, the healing process may interfere with blood flow.

9. No bleeding may be performed from a damaged eye. If both eyes are damaged, eye bleeding must cease.