

Science Education Collection

Compound Administration IV

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Overview

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There are many commonly used routes for compound administration in laboratory mice and rats. Protocols may, however, require the use of the less commonly used routes: intracardiac, footpad, and retro-orbital injections. Specialized training is essential for these procedures to be performed successfully. Justification for these routes may need to be provided to gain Institutional Animal Care and Use Committee (IACUC) approval.

Principles

Intracardiac administration has been used in a variety of applications, including the development of an animal model of bone cancer metastasis, as well as an examination of the effects of direct intracardiac delivery on the outcome of myocardial infarction. This procedure is often done through the use of an ultrasound to guide the needle into the correct location in the heart.² However, when performed correctly utilizing the proper landmarks, this procedure can be performed without the use of ultrasound visualization.

Due to the invasive nature of the procedure, the use of intracardiac injection must be scientifically justified in an IACUC protocol. Only one survival injection should be permitted. This procedure requires the use of a general anesthetic, either inhalant or injectable, as per the guidelines established within an organization. Needle selection should be the smallest size possible that will allow for the viscosity of the material injected; generally, a 27-30 gauge needle is used. Injection volumes range from 100 μ L to a maximum of 300 μ L.

Intravenous injections in the tail of mice are both challenging and often unsuccessful. An alternate route of intravenous administration is through the retro-orbital plexus. While this technique necessitates training and skill to perform, studies have shown that there is a higher success rate with the retro-orbital injection than with lateral tail vein injection.^{3,4,5} Anesthesia is required to prevent the mouse from moving during the procedure. General inhalant anesthesia delivered either via a bell jar or an induction chamber attached to a precision vaporizer is effective. However, if inhalant will be used, be aware that the animal will begin to recover quickly once it is removed from the chamber, so one must be ready to perform the injection. A topical ophthalmic anesthetic (tetracaine or proparacaine) is recommended when multiple injections are to be performed.

The orbital venous structure of the mouse and rat are different. The mouse has a sinus or convergence of several vessels, including the supraorbital vein, dorsal nasal vein, the inferior palpebral vein, and the superficial temporal veins that fill the space in the orbit around the eye. In the rat orbital area, there is a network or plexus of vessels. As with all injections, the needle selected should be the smallest size possible; generally a 27-30 gauge needle. Although there have been reports of larger volumes, the maximum volume is 150 μ L per eye.^{3,4,5} One injection per eye, per day, is recommended, with a total of two injections per eye for survival procedures. Also, there should be at least a one-day interval between injections. For a nonsurvival procedure, volumes up to 500 μ L can be administered.

Despite the controversy, the use of the foot pad as an injection site is still required for some studies. It has been demonstrated that when injected via the foot pad, the antibody response in some mouse strains was significantly stronger than when injected into the hock.⁶ All animals must be closely monitored for signs of pain, level of food consumption, and for normal ambulation. Self-mutilation of the foot can occur to the extent of the foot being destroyed. This is a sign of chronic pain. Any animal demonstrating self-mutilation should be called to the attention of the veterinary staff immediately.

Footpad measurements should be done daily as soon as obvious swelling has occurred. Endpoints must be in place according to IACUC guidelines. Generally, the animal must be euthanized when the lesion or tumor interferes with the animal's ability to ambulate or reach food and water. The maximum volume that can be injected into a footpad is 50 μ L. A 29-30 gauge needle is recommended for the injection.

Procedure

1. Intracardiac injection

1. Landmarks and positioning: Position the mouse or rat either in right lateral recumbency (with the left side facing upward) or in dorsal recumbency, and identify the landmarks.
 1. Position the heart approximately level with the point of the elbow and just to the left of the sternum.
 2. Insert the needle between the ribs at the point of the elbow.
 3. In an animal in dorsal recumbency, insert the needle into the chest parallel to the table.
 4. In an animal in lateral recumbency, insert the needle into the chest perpendicular to the table.
2. Injection of the article
 1. Ensure there is minimal movement of the needle once it is inserted into the heart to prevent the muscle from being torn and causing bleeding into the pericardial sac, as this compromises cardiac function.

2. Aspirate the syringe to determine proper placement. There is often a flash of blood into the hub of the syringe.
3. Hold the syringe so that the hand is not repositioned on the syringe for injection once the needle has been positioned in the heart.
4. Inject the article in a slow and steady manner.
5. Withdraw the needle slowly to minimize bleeding.
6. Mice that experience respiratory distress, seizure, cyanosis, prolonged ataxia, or other sequelae related to injection trauma must be evaluated by the veterinarian staff and euthanized according to their recommendation.



Figure 1. Intracardiac injection in mice.

2. Intravenous injection utilizing the retro-orbital plexus

1. Injection landmarks and positioning
 1. Place the animal on a flat surface in lateral recumbency.
 2. Insert the needle into the medial canthus of the eye at a 45° angle to the nose.
 3. The needle depth must be sufficient to penetrate the conjunctival tissues and advance behind the ocular orbit and into the ocular plexus. When placing the needle, it should not encounter the bone at the back of the orbit.
 4. Place the index finger on the top of the head with the thumb on the jaw.
 5. Gently pull back and downward to tighten the skin and protrude the eyeball.
 6. Take care not to apply pressure on the trachea and restrict air flow.
2. Injection
 1. Place the needle behind the eye and not intra-ocularly.
 2. Ensure that the needle has minimal movement once inserted into the retro-orbital plexus, or the vessels will rupture causing bleeding and loss of the agent into the tissues behind the eye. Hold the syringe so that the hand is not repositioned on the syringe for injection once the needle has been positioned in the retro-orbital plexus.
 3. Do not aspirate with the syringe, as you will collapse the vessels.
 4. Inject the article in a slow and steady manner.
 5. Withdraw the needle and apply light pressure to the eye to control bleeding and to provide hemostasis.
 6. Mice that experience swelling, conjunctivitis, or other sequelae related to injection trauma must be evaluated by the veterinary staff and treated or euthanized according to their recommendation.



Figure 2. Retro orbital injection in mice.

3. Footpad Injection

1. For restraint, place the animal in a restraint tube with one hind foot isolated, and extend by grasping the skin above the stifle.
2. Wipe the foot with water or alcohol to remove debris prior to injecting.
3. Inject subcutaneously into the center of the hind foot forming a small bleb at the injection site. To avoid the blood vessel that runs the length of the foot, inject just off the midline parallel to the vessel.

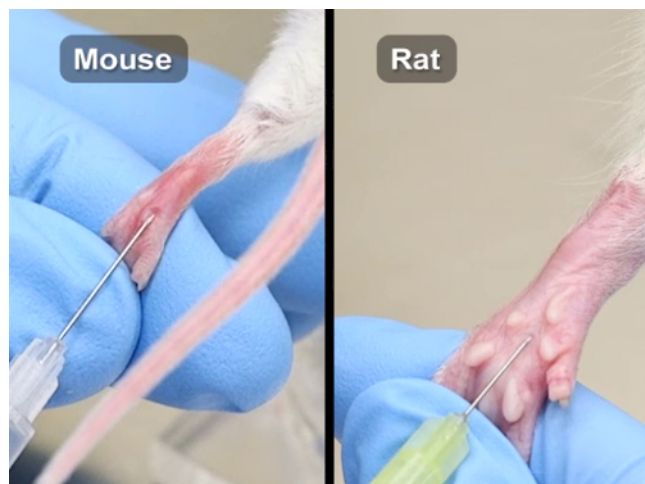


Figure 3. Footpad injection in mice and rats.

Summary

The administration of compounds into animals can have a significant effect on both the wellbeing of the animal and the outcome of the experimental data and scientific value. The proper method of delivery is essential to the success of the experiment. Many factors must be considered to determine the best route, including the scientific aim of the study, the pH of the substance, the required dosage volume, the viscosity of the substance, and the wellbeing of the animals. Technical expertise is also a requirement for all injection methods.

References

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