University of Notre Dame Institutional Animal Care and Use Committee
Freimann Life Science Center
Policy for Survival Intra-cardiac Injection in Rodents

Purpose

Intra-cardiac injection is considered to be a terminal procedure in most instances. Exceptions include creating metastases in the lungs, spine, brain, and other body regions with the injection of cancer cells, visceral infection with blood parasites, and emergency drug delivery. All survival cardiac punctures require scientific justification and observation for proficiency by FLSC veterinary staff. When survival cardiac puncture is performed, the following procedures and precautions are designed to minimize mortality and increase animal welfare.

Equipment

1 cc syringe with 30 - 27g x ½” needle (mice)
1 cc syringe with 30 - 26g x ¾” to 1” needle (rats)
Paper tape
Cotton tipped applicator stick or 2” x 2” gauze square
70% isopropyl alcohol
Sterile cell suspension, infective parasites, or drug

Procedure

1. All survival intra-cardiac injections must be done under general anesthesia. Injectable anesthetics or inhalation anesthetics can be use either alone or in combination.
2. The animal is anesthetized and positioned in dorsal recumbency on an insulating pad or platform.
3. The hair on the animal’s chest is wetted with alcohol. Be careful not to saturate the animal. It is best to use a cotton swab or a gauze pad to apply the alcohol to focus the application area.
4. The forelimbs are taped to the pad/platform perpendicular to the midline.
5. A piece of tape can be placed horizontally across the abdomen above the hips to steady the animal and avoid movement once the needle has been inserted.
6. Prepare the syringe using a maximum of 100 microliters of injectable material. A small bleb of air can be positioned between the plunger and the injection material. This air allows for a pulsing of blood into the syringe to help confirm correct placement into the left ventricle.
7. To make the injection using the ventral approach:
   - Locate the xiphoid and the manubrium sternum.
   - Find the midpoint between the xiphoid and the manubrium sternum.
   - The needle insertion point is 1-2 mm to the left of the midline at the midpoint identified above.
   - Direct the needle vertically to the injection point (perpendicular to the plane of the pad/platform).
   - The syringe and needle must be perfectly perpendicular at the insertion point.
   - The hand must remain completely steady once the needle has been inserted or laceration of the heart can occur. Brace the hand on the table or your other hand.
   - Insert the needle to the depth of about 2 mm.
8. To make the injection using the lateral approach:
   - Locate the position on the chest wall that is even with the elbow when the foreleg is flexed against the chest.
   - Using your fingertip palpate the location where the heart beat is the strongest.
   - Hold the needle parallel to the surface of the table and perpendicular to the chest wall.
   - Direct the needle into the heart through the 2nd or 3rd intercostal space.
   - The syringe and needle must remain completely perpendicular to the insertion point.
   - The hand must remain completely steady once the needle has been inserted or laceration of the heart can occur. Brace the hand on the table or your other hand.
Insert the needle about one quarter the width of the chest.

9. Check for proper placement by applying a very slight back pressure to the plunger. Bright red oxygenated blood should enter the syringe hub.

10. Inject the material into the heart. The injection must be done slowly and steadily over the course of 30 to 60 seconds. DO NOT inject the air bubble in the syringe. More rapid injection can result in clumping of the cells and clogging of the arteries, a shock to the system since most injectable materials have been kept on ice and are very cold, or can cause expansion of the ventricle and disruption of the heart rhythm.

11. Once the material has cleared the syringe, carefully remove the needle from the heart. Damage to the heart muscle can occur if the needle moves laterally during extraction from the chest.

**Post-procedure Care**

1. Immediately after the procedure, release the tape from the forelegs and abdomen.

2. Place the animal in sternal recumbency in a clean cage with sufficiently deep bedding to act as an insulating layer.

3. Place half the cage on a heating source and orient the animal on the heated side of the cage.

4. The primary complications are piercing the lungs resulting in bleeding, needle stick injury to the coronary arteries, hemorrhage within the pericardium, and tamponade (constriction of the cardiac blood vessels). The probability of complications can be reduced by good injection technique and using a small gauge needle. Observe the animal for any of the following signs immediately:
   - Dyspnea (difficulty breathing)
   - Cyanosis (skin and membranes have a bluish color)
   - Respiratory sounds especially gurgling or a rattling
   - Bloody discharge from the nose or mouth
   - Ataxia (inability to coordinate voluntary muscle movements — staggering)
   - Hindquarter paralysis
   - Head tilt

5. During the anesthesia recovery period make observations every 5 to 15 minutes. An observation log must be maintained for animals undergoing this survival procedure and be available for IACUC inspection.

6. Observations must be made hourly after anesthetic recovery for an additional 6 hours.

7. Euthanasia criteria will include the following:
   - Dyspnea or labored breathing especially mouth breathing
   - Persistent cyanosis beyond the anesthesia recovery period
   - Ataxia or disorientation beyond the anesthesia recovery period and does not resolve within 4 hours of injection

**References**

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