eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Fluid and Drug Administration Procedure Animal Model in Biomedical Research

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ARTICLE INFO

Keywords:
Fluid
Drugs
Administration

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v5i8.312

ABSTRACT

Drugs, vaccines, injectable anesthetics or other agents are to be administered, one or more of several different routes may be selected. The routes selected are governed by the nature of the agent being administered, the animal, the purpose of the administration and other factors.

1. Introduction

When drugs, vaccines, injectable anesthetics, or other agents are to be administered, one or more of several different routes may be selected. The routes selected are governed by the nature of the agent administered, the animal, the purpose of the administration, and other factors. The more common routes of administration used for laboratory animals are classed as follows:

Guidelines for fluid and drug administration gastro-intestinal tract:

- Oral or per os (PO) - through the mouth
- Gavage - into the stomach via tube Parenteral
- Intravenous (IV) - directly in the vascular system through a vein
- Intraperitoneal (IP) - injected into the abdominal cavity
- Subcutaneous (SQ) - injected under the skin
- Intramuscular (IM) - injected into a muscle
- Intradermal (ID) - injected between the layers of the skin.

Gastro-intestinal tract

Substances may be admitted orally by addition to the food or drinking water, by use of a capsule or pill or by instillation into the mouth using a mechanical device, such as a syringe. Capsules or coated pills are rarely used in rabbits or rodents. When used, capsules or pills are placed in the mouth near the back of the tongue, and the animal is induced to swallow by stroking the throat. Stomach tubes or gastric feeding needles are inserted through the mouth into the stomach or lower esophagus. Care must be taken that the tube does not enter the trachea or the needle puncture the esophagus. In most cases, introduction of the tube toward the rear of the

mouth will induce swallowing and the tube readily enters the esophagus. A violent reaction (coughing, gasping) usually follows accidental introduction of the tube into the larynx or trachea. Flexible or plastic tubes may be bitten or chewed and some care must be taken to prevent this. With rabbits, a dowel of wood or plastic with a hole in the center is inserted behind the incisors. This prevents chewing and permits easy entrance of the stomach tube. Rabbits should be placed in a restraining device before attempting this procedure to avoid unnecessarystruggling and injury. A small, curved, metal tube, usually with a ball on the end (feeding needle) is often used with small rodents. Entrance may normally be obtained without anesthesia using ordinary hand restraint and the ball prevents trauma to the esophagus and oral cavity. With the stomach tube fittedto a syringe or aspirator, material may be administeredor withdrawn as required. A safe volume to gavage ratsand mice is 10 ml gavage solution per kg body weight.

Parenteral routes of administration involve injections into various compartments of the body. Sites used for collection of blood from veins may also be used for intravenous administration. Intraperitoneal administration is one of the most frequently used parenteral routes, but other commonly used locations are the musculature and the subcutis. Materials given intramuscularly must be small in amounts. Absorptionvia this route, however, is more rapid than subcutaneous administration. Regardless of the route to be used, it is essential that the subject be securely restrained to avoid injury to personnel, caused bydislodged needles, and to animals because of struggling. The investigator should know the physiological properties of the substance for injection. Considerable tissue damage and discomfort can be caused by irritating vehicles or drugs. The use of the rabbit foot pad as an injection for antigens, with or without adjuvant, is expressly prohibited since it is a needless and painful procedure. A more suitable site for antigen injection is subcutaneously or intradermally over the dorsal body trunk. In general volumes must be limited to a maximum of 0.1 ml per Intradermal or 0.25 ml per subcutaneous injection site.The following outline provides basic information on equipment and techniques for parenteral injections in rodents and rabbits.

Mouse intravenous

Equipment - 27-30g needle, 1 ml syringe, mouse holder, warming lamp. The lateral veins of the tail are the most frequently used veins. Best results are obtained if the tail is immersed in warm water or the mouse is warmed in the cage with a warming lamp. Theveins can be seen when the tip of the tail is lifted and rotated slightly in either direction. The tip of the needlecan be followed visually as it penetrates the vein. Trialinjection soon discloses whether or not the needle is inthe vein. Practice and training are essential. This is notan easy technique to master quickly.

Intraperitoneal

Equipment - Syringe and 23-27g 1/2 to 1 inch needle, preferably with a short bevel. The injection is made in the lateral aspect of the lower left quadrant (Figure 2). The use of a short bevel needle and its insertion through the skin and musculature followed by immediately lifting the needle against the abdominalwall aids in avoiding puncture of the gut lumen. Rapid injection with a large syringe may cause bruising of tissue and hemorrhage from the pressure of the spray and should therefore be avoided. Unless the left leg is immobilized, there is considerable risk of the mouse's movement causing puncture of the viscera. The maximum volume injected IP into a 20 gm mouse should not exceed 2 ml.

Intramuscular

Equipment - 26-30 g, 1/2 inch needle with 1 ml syringe. The back and hind leg muscles are the usual sites selected. Due to the small muscle mass available, the volume of drug injected should be limited. Subcutaneous: Equipment - 25-27 g, 1/2 to 3/4 inch needle and 1 ml syringe. The site usually chosen is thearea between the shoulder blades. This route is usefulfor the administration if isotonic replacement fluids (0.9% saline) in the dehydrated animal.

Retro-orbital injection of mice:

Materials:

Anesthetic that is described in investigator’s protocol Insulin syringes with 28 g. needles Fluid to beinjected

Procedure:

1. Anesthetize mouse.
2. Place mouse in lateral recumbency with its feet facing you. If needed, place mouse's head on a stable surface that is slightly elevated from the tabletop. The medial canthus of the eye should be on thesame level as the needle of the syringe. Having the head elevated makes it easier to advance the needlein a straight line rather than at a downward angle as when the head is resting on the table top.
3. Grasp mouse firmly at scruff of neck until eyes bulge slightly. This is the same restraint method asis used for retro-orbital bleeding.
4. Rest your wrist on the tabletop in front of themouse's head. Hold the syringe between your thumband 1st or 2nd finger with the bevel of the needle pointing up.
5. Moving your hand in a steady motion, advanceneedle parallel to the mouse's nose and insert it at the medial canthus into the space between the eye and the surrounding tissue. Insert needle until youfeel a tiny "pop" or change in pressure as it punctures the connective tissue surrounding the globe.
6. Inject no more than 50 microliters (0.05 ml) of fluid into the space. If done properly, you will not see any fluid leaking around the eye. If you do, aspirate the fluid without removing the needle, redirect needle slightly, and repeat injection.
7. Remove needle with smooth steady motion.

Rat intravenous

Equipment - Depending upon the size of the rat, needles as large as 20 g may be used. One half to one inch length needle is used. A rat holder and warming lamp are also important. The techniques described forthe mouse also apply here. Confinement within a cylindrical holder is the usual method for restraint. Light anesthesia with ketamine and Xylazine is helpful for restraint. Prolonged intravenous administration/sampling may be accomplished by jugular vein catheterization.

Intraperitoneal

Equipment - 23-25 g, 5/8 to 1 inch needle. The location is the same as described for the mouse. Restraint is best accomplished with a second person holding the rat in a head-down, stretched-out position, or with light anesthesia. Intramuscular: Equipment - 25-26 g, 1/2 to 5/8 inch needle with 1 ml syringe. The back and hind leg muscles are used. Subcutaneous: Equipment - 23 g, 1 inch needle. The usual site is between the shoulder blades. Be sure and use adequate restraint. Rat skin is thick and difficult to penetrate. Care should be taken to avoid accidental human injections.

Rabbit intravenous

Equipment - 22-25 g, needle or butterfly catheter of suitable length with a syringe. A rabbit holder of plastic or metal construction is necessary. The marginal ear vein is used almost exclusively for substance administration (Figure 3). Place the rabbit in a rabbit holder. The hair over the vein is clipped or plucked and the skin cleansed with alcohol. The vein may be distended by flicking with the finger a few times. Holding off the 32 vein near the base of the ear will alsohelp distention. Refer to the next section for blood collection techniques.

Intraperitoneal

Equipment - 20-22 g, 1 to 1 1/2 inch needle with suitable syringe. Smaller needles may be used for small volumes of low viscosity substances. An assistant or chemical restraint is necessary to reduce motion of the limbs. The abdomen is clipped and the skin disinfected. The rabbit is held in a head-down position. Injections are made in the lateral aspect of the lower left quadrants. Caution must be taken to avoid puncturinga distended urinary bladder, the bowel or the liver.

Intramuscular

Equipment - 22-23 g, 1 inch needle. The most frequently used sites are the back muscles lateralto the vertebrae and caudal to the Aab, or the lateral thigh musculature. Volume should not exceed 0.25 mlper site for adjuvant and antigen combinations. If repeated injections are to be made, rotate sites. Adequate restraint is important.

Subcutaneous

Equipment - 20-23 g, 1 inch needle. They are most frequently used is between shoulder blades. Volume should not exceed 0.25 ml per site for adjuvant and antigen combinations.

Intradermal

Equipment - 25-26g, 1 inch needle. The most frequently used site is over the shaved back. Volumes should not exceed 0.05 ml per site for adjuvant and antigen combinations. Injection sites should be spaced3-4 cm from each other to prevent confluence.

Blood collection

Site preparation

Certain general procedures and precautions are applicable to methods of blood collection as well as to administration of fluids and anesthetics. When venipuncture is required, hair should be shaved from the site for better visibility. The area of injection should be cleansed with alcohol. Some procedures will require anesthesia; others may be carried out without anesthesia, provided suitable restraint is possible. In order to visualize veins better, one of several methods of dilation may be used. The vessel may be occluded with digital pressure to cause enlargement. Heat will also cause dilation. When using the rabbit ear, or mouse or rat tail, a low watt light bulb may be used for heat. The preferred method of collection of large volumes of blood from the rabbit ear is with the use of a droperidol-fentanyl tranquilizer that promotes arterial dilation and makes blood collection from rabbits simple for even the inexperienced phlebotomist.

Guidelines for blood collection Equipment needed:

Needles of appropriate gauge and length must be selected with care. For the tail vein or artery of rats and mice, small needles (25-30g) should be used. For other vessels in other animals, the suitable size will depend upon the size of the animal and vessel. Technique: Proper insertion of the needle into the vein or artery isthe most tedious part of the procedure. Certain guidelines may be given, but only practice can beexpected to provide any proficiency. A precise, careful introduction of the needle is always best. The needle isinserted parallel above the vessel and the tip directed into the lumen along with the longitudinal axis. The intracardiac puncture generally represents the most practical method of blood collection from small rodents when more than a few drops are required. It is also useful in rabbits for exsanguination. Animals must be anesthetized and placed in dorsal recumbency. The point of the strongest heart beat is determined with the forefinger. The needle is inserted through the skin, between the Aab at this site, directly into the heart. Blood should be withdrawn slowly.The cardiac route forblood collection is a terminal procedure. In the rabbit, the marginal ear vein is most useful for intravenous injection, but not blood collection.

Blood collection is best accomplished from the central ear artery via butterfly catheter or needle. Thirtyto forty ml may be collected in this manner. The absolute maximum of blood to be withdrawn at one time is 9 ml/kg body weight. The PCV (packed cell volume) must be measured at each collection if such large volumes of blood must be withdrawn. If the PCV drops below 35%, collection must be reduced. The useof a droperidol-fentanyl tranquilizer promotes arterial dilation, relaxes the rabbit and makes blood collection from rabbits simple for even the inexperiencedphlebotomist.

In the rodent, blood collection by cutting off toes is not permitted. Collection from the tail artery may be increased by warming it in water. Animals should be restrained in restraining device or anesthetized. After cleaning, a small nick is made on the ventral midline ofthe tail and blood is collected. Digital pressure will stop the blood flow. Withdrawal of blood from the orbital venous plexus of rats and orbital sinus of mice and hamsters is frequently used. When bleeding the mouse, hamster and rat by the retrobulbar technique, the hematocrit capillary tube is directed toward the major venous structures of the orbit. Knowledge of the location of the venous structures and the technique is essential. Anesthesia is required for all retrobulbar bleeding procedures.

Maximum blood volume for survival collection in lab animals. The maximum amount of blood to be collected, as a survival procedure, from the following laboratory animals is 15% of the circulating bloodvolume. Frequency of collection should not exceed every other week. Hematocrit must be monitored and fluid replacement considered for protocols which require blood collection in larger volumes or at more frequent intervals.

Figure 1: Rodent gavage needle parenteral.

Figure 2: Intraperitoneal injection of the rat.

Figure 3: Marginal ear vein of the rabbit.

Figure 4: Central ear artery of the rabbit.

Figure 5: Retrobulbar blood sample collection in the mouse.

Species	**Total blood volume**	**15% blood volume**
Rabbit	60 ml/kg b.w	9.0 ml/kg b.w
Rat	58 ml/kg b.w	8.7 ml/kg b.w
Mouse	78 ml/kg b.w	11.7 ml/kg b.w
Dog	90 ml/kg b.w	13.5 ml/kg b.w
Cat	66 ml/kg b.w	9.9 ml/kg b.w

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