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A Review of Parenteral Administration of Testing Substance in Rabbit and Mice

Abstract

There are many publications that deal with the administration of test substances to animals and the removal of blood samples for different objectives. The parenteral route of drug administration includes further different routes, i.e., drug administration through muscles (IM route) through veins (IV route) and through subcutaneous route. We can manage to check the outcomes of test substances within minutes when we go for the IV route as in this route; the drug directly goes to the systemic circulation. Different experimental animals are utilized to check the possible outcomes of test substances that include Rabbit, mice, and rat. Various factors like dose strength, dose frequency and the meaning by which drug can be administered to an animal can be examined. The emphasis on drug safety should be the main priority while injecting drug substances. Animal ethics guidelines should be followed in this regard. AEG basically provides us with safety guidelines that should be followed for safe handling.

Key Words: Intramuscular, Anesthesia, Doses, Administration, Mice, Subcutaneous, Parenteral

Introduction

To meet our essential regulatory demands, experimental animals play a vital role as their dosing is compulsory for several scientific investigations. To check the safety margins of new drugs, it is important to check the effective doses by having trials on experimental animals. When chemicals have low toxic properties, or they are poorly soluble, we must use larger volumes to entertain scientific as well as regulatory requirements.

Now administering different substances to experimental animals needs a lot of careful experience. The focus should be on maximum positive outcomes and minimum relative adverse events. There are a lot of routes to which a drug can be administered to the experimental animals, but our main focus would be on the parenteral route of drug administration. ([Lee et al., 2019](#))

The parenteral route has further several routes by which the drug would be administered. The intravenous route is where the drug is directly delivered to the blood by puncturing the vein of the animal. The intra-arterial route involves drug delivery

through the arteries of animals. The Intraperitoneal route is linked with drug delivery through the abdominal cavity. Subcutaneous administration involves drug delivery through the skin of the animals. Now when we place the drug between the two layers of skin of an animal, it would be called the intra-dermal route of drug administration. When the drug is delivered to muscles, it would be the intramuscular route of drug administration. The most efficient way of drug delivery to animals is the intracutaneous route of drug delivery, as it avoids the requirement of solute absorption. Here the drug is administered in the form of bolus or infusion directly to the blood vessels. ([Glenny, Buttle, & Stevens, 1931](#))

When we talk about pharmacokinetic studies and different pharmacokinetic properties in animals, the one route of drug administration that is widely used in mice and other rodents is the intraperitoneal route of drug administration. While in the case of rabbits, the most frequently used route is the intravenous route of drug administration. While we

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apply any of the routes of drug administration, the focus should be on proper control of the animals, as these animals should be restrained efficiently to stop useless struggles by the animal. All the needles used should be sterile. The drug given to the animal should not cause discomfort to the animal, or any of the technique that causes harm or discomfort to the animal should be strictly prohibited. ([Richard et al., 2011](#))

Factors Affecting a Parenteral Route

When the drugs are administrated to animals from the parenteral route, different factors play a vital role in positive output. These include the volume of the dose used, checking the stability of substance, pH value, viscosity, osmolality studies, sterility studies of the formulation and biocompatibility studies. The needle size should be the smallest in the parenteral route for better outcomes.

Vehicles for Drug Administration

The selection of vehicle for drug administration in animals is an important factor. Vehicles should be selected that follow specific criteria, i.e., and it should not affect the biological activity of drugs, which means it should be biologically inert. It should have no harmful effects on the animals.

The most widely used vehicles are some isotonic solutions, co-solvents, certain oils, and some buffered solutions. When we have to administer drugs in the form of suspensions, their viscosity and PH should be checked accurately. ([Vajanto et al., 2002](#))

Requirements for Parenteral Administration

1. Animal restrained
2. Razor
3. Gloves

4. Warning lamps with bulbs of 40 to 100 watts
5. The needle of 27 to 30 gauge and 1 ml capacity for mouse
6. The needle of 22 to 25 gauge for rabbits
7. The needle of 20 gauge for rats
8. Alcohol pad
9. Cotton

Parenteral Administration to Rabbit

Intravenous Administration

Here marginal ear vein of Rabbit is used to deliver the drug substance. The procedure followed in this specific category is as follows:

1. An animal should be placed in a restrainer carefully.
2. The hair over the marginal ear vein is removed carefully.
3. The injection site at the ear should be cleaned with an alcohol swab.
4. Now insert the needle with a gauge of 22-30 size with syringe or here butterfly needle can also be used.
5. After inserting the syringe in the ear vein, pull the plunger of the syringe back with pressure. If there is a flash of blood present, it shows that the syringe is not properly placed.
6. Now administer the drug substance in steady-state motion.
7. Take an alcohol pad and apply it to the withdrawal site with pressure so that bleeding can stop. ([Shyu, Manor, Magner, Yancopoulos, & Isner, 1998](#))

Now there are some considerations that are followed in IV administration of the drug in rabbits, which includes this process has a rapid absorption rate. It can be easily performed by using a general type of anaesthesia. The volume should not be more than 1% of the total body weight of Rabbit.

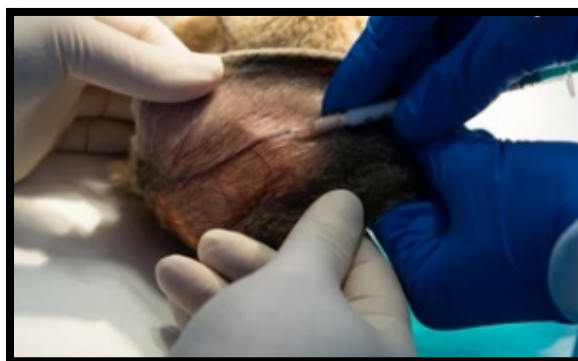


Figure 1: Intravenous Injection in a Marginal Vein

To dilate the blood vessels before injection, the ear should be massaged for a minimum of 30 to 60 seconds, or a warm compress should be applied to the ear. Devices to be used should be sterile, and they should be discarded after a single-use. Performers should wear gloves, and they should wash their hands properly after performing the whole procedure. ([Evans, Mazzocchi, Nelson, & Rubash, 1984](#))

When the following factors are noted during the procedure, we must contact a qualified veterinarian, these includes:

1. Swelling or pain at the site of injection
2. Stiffness of the vessels
3. Infections at the injection site

Table1. The Blood Volume in Different Animal Species

Blood volume circulating in laboratory animals (Blood volume in ml per kg)		
Species	Recommended Mean	Range of means
Rabbit	56	44-70
Mice	72	63-80

Intramuscular Injection to Rabbit

In this category, the drug substance is injected into the thigh muscles. This route of drug administration may be painful as muscle fibres got disturbed by drug substance material. There are high risks of nerve damage in this category, so the site should be chosen with great care. Oily formulations can be injected by this route as these formulations remain in depot form in muscles for more than 24 hours. Candal injections should be avoided as they can damage the sciatic nerve. Now IM injection involves the following steps:

1. The animal should be restrained manually.

2. The injection site should be cleaned by alcohol swab.
3. The needle should be cleaned and inserted in such a manner that it should not harm the sciatic nerve.
4. The syringe plunger is pulled back with pressure; if there are some fleshes of blood, it shows improper placement.
5. The drug substance should be administered within an optimum range, as too rapid administration can lead to problems.



Figure 2: Intramuscular Injection in Thigh Muscles

Here there is no need for general anaesthesia, but the animal should be restrained properly. This type of injection is common for different antibiotics and sedative drugs. Injection volume here should be

0.5 – 1 ml per site. The needle should be inserted at a 90° angle for IM injections. After injection, hands should be washed properly, and gloves should be discarded. ([Miyazaki et al., 1995](#))

Table 2. Sites of Animals for Repeated Blood Sampling

Sites Suggested for Repeated Blood Sampling	
Species	Suggested sites
Rabbit	Marginal ear, central ear artery and jugular vein
Mice	The saphenous or lateral tail

Subcutaneous Injections

These types of injections are injected into the loose skin near the neck region. It forms a space underlying between the muscles and the outer skin. This route of drug administration is not suitable for irritant drugs as it can lead to severe skin problems. This route is used commonly. It can be observed without the use of general anaesthesia, but proper handling is very important for adequate administration of the drug to an animal. The needle should be placed at an angle of 45°. The process of injecting the drug material into an animal should be smooth, and there should be no harm to the animal in any way. Now the process for proper subcutaneous injection procedure involves the following steps:

1. To restrain the animal is the most important step in the subcutaneous administration of the drug to the animal. It should be restrained on the table.
2. The injection site should be cleaned with an alcohol pad.
3. The needle should be inserted near the skin of the neck between the outer skin and the muscles.
4. The plunger of the syringe is pulled back with force; if there is any flesh of blood, the needle is not properly maintained.
5. The drug substance should be administered in a steady motion. [\(Bonanomi, Velvart, & Weder, 1987\)](#)



Figure 3: Subcutaneous injection in loose skin

Parenteral Administration in Mice

The most common animal used in the administration of the drug in mice, so a policy should be followed, which promote positive results and decreases the discomfort of the animal. There are certain chemicals that are toxic, so their handling as well as route should be determined for better results. The policy had been designed which promote the success of the

experiment, and the thing needed to be considered included. Sterility, pH, Physical and chemical characteristics of the drug, how many doses we have given to animal etc. The principal investigator basically determined the doses being given to mice, and it should be in mg/kg. [\(Kim, Han, Kim, & Kim, 2010\)](#)



Figure 4: Parenteral Administration to Mice

Parenteral Routes in Mice

It mainly depends on the extent of the response we want. The person performing the experiment should fully know about the chemicals and vehicles he was using because the chemical may cause irritation and may be toxic for the animal. The place where medicine should be injected should be determined accurately by bringing the plunger back. If there is blood on pushing back, then it is correctly placed, and if there is fluid, then there is an error. Several parenteral routes are for the mice; the first is subcutaneous, in which mice are put in a normal restrainer, or it is properly handled, and with one hand skin pouch that is loose skin on the top is being stretched, and injection is being placed. ([Dupuis, McDonald, & Ott, 1999](#)) Pellets are used for long term effect in the case of intraperitoneal injection; no anaesthesia is required. One person holds the mice while the other puts injection, so the absorption is comparatively fast. The intravenous and intra-arterial route is commonly known as an intravascular route. In the case of these routes, two veins are preferable tail vein and the jugular vein. In the tail, vein anaesthesia is not required, while in the jugular vein, there is a need for anaesthesia. In the case of irritating substance, there is a need that injection should be diluted prior to injecting. In the case of retro-orbital injection in mice, anaesthesia is administered. ([Briner,](#)

[Kuo, Keating, Rogers, & Greenstein, 1993](#)) It is the most complicated type of route because it may lead to the tumor, so to avoid this, they must be shaken prior to administration. This injection is administered to the retro orbital sinus. ([Xue et al., 2012](#)) Next is intramuscular, in which the injection is administered directly to muscles, but in mice, it is not administered due to lack of muscular mass, and there are chances of sciatica if they are not administered properly in the gluteal muscles. In the case of intradermal injection, there is a lack of inflammation and immunologic studies, which is why it is not administered. If the safety policy approved, then it is administered. In the case of ocular injection, it is very limited and only use for certain compounds. There is need of intranasal injection and volume should not exceed from 30 microliter in one nostril. Another route is intra tracheal route in which mice is placed in vertical position and volume injected is 50 microliter and head are in upward direction with tongue out and the injection is aspirated. Next is intracranial injection in which either bolus is given or there is continuous administration. It can be done via surgery or direction injection. Next is intrathoracic administration in which injection is in thoracic cavity i.e. in chest with aid of anesthesia. ([Israelit, Long, White, & Hull, 1973](#))



Figure 5: Intramuscular Injection in Thigh Muscles

Intra cardiac injection is administered in chest cavity under anesthesia. The needles used for all injection should be small and sterile and one needle should be used for one injection. The number of doses given to mice should be minimum, try to administer only one injection in one day because the analgesic has very limited duration of time. Beside needle the PH plays important role because the injection are directly placed in blood so PH should be neutral because it may lead to serious injury. ([Koshkina, Waldrep, Seryshev, Knight, &](#)

[Gilbert, 1999](#)) The odor and taste are not of much importance because they are injected in blood vessels and that is main advantage of parenteral administration. The solution or vehicle in which drug is diluted should be iso-osmolar that is lactate ringer and should be injected with much attention. Some injectable compounds are potent so they must be diluted by using different vehicles. Similarly, some injectable compounds are in powdered form, so they are also converted to solution by adding vehicle i.e., PBS, DMSO, water, comoil etc. Beside this emphasis

should be made on storage and placement because they should be placed in special place in which there is no sunlight because most of them are degraded by

direct sunlight. ([Usach, Martinez, Festini, & Peris, 2019](#))

Table3. Route and Volume for Experimental Animal

Route and Volume (ml Per kg)			
Species	IP	IM	IV
Rabbit	5	0.25	2
Mice	20	0.05	5

Injection Volume

Different volume is administrated for different injections, e.g., for subcutaneous injection, the site is flank or the skin mass is more, and about 1-2ml is administered for the subcutaneous. Similarly, in the case of IM, the injection is placed in gluteal muscles, perpendicular to the muscles and volume should be 0.03-0.05ml, and in the case of intraperitoneal injection, as the injection is placed in the peritoneal cavity that in the lower right quadrant, so the amount of injection is less than 2ml. Similarly, in the case of IV injection, the injection should be placed in a lateral vein, and in IV infusion, the solution is placed through the cannula. In the case of retro-orbital injection, it should be placed in the sinus with a volume of 180-200 ml with 29-gauge needles. In the case of intracranial injection, it is placed in the cranium with the help of a cannula, and its needle is not available. The parenteral route of administration had many advantages, like they can be used for patients who are continuously vomiting. They can be used for a substance having an irritating taste. They also had very high bioavailability and rapid effect as compared to oral substances. ([Barondes & Cohen, 1968](#))

Method of Administration Through a Different Route

First of all, mice should be handled properly, or they are kept in a restrainer or cage. There should be a proper lightening system. They should be left for something in order to get rid of anxiety; then their tail moves around in order to see the vein properly. Once the vein is clearly seen then the needle is injected into the tail vein, the plunger is pulled back. If there is blood, then the needle is not placed properly, and the second indication of improper placement is the production of the bleb, which ultimately leads to chronic disease. We can inject to another side of the needle that is not at its correct position. We can inject at least five sites on the tail vein once the injection is placed properly, then put out the needle and clean the site properly. In the case of intramuscular injection, the injection is placed in the gluteal or thigh muscle.

These mice should behold after correctly holding the mice, and then the injection is placed at muscle at 90° or 45°, and we have to check if the injection is placed correctly by pulling the plunger back. In intraperitoneal injection, the mice should be placed properly in a resting position that is in restrainer, or it can be held by one person, and injection should be placed by other person. (F. [Wu et al., 2012](#)).

For injecting intraperitoneal injection, the mice should be held properly, that is, his head is facing in a downward direction and injection is administered in the parallel direction in abdominal region that is rich of blood vessel that is third quadrant. In order to check if the injection is properly placed or not the plunger is move back if there is blood on pushing back the plunger then it is not correctly placed. ([Engineering, 1994](#))



Figure 6: Intraperitoneal Injection in the Lower Abdominal Region

In the case of subcutaneous injection, it is placed on the loose skin of mice. First of all, the loose skin is stretched to make a pouch, then the injection is placed at a specific angle. There is certain precaution regarding this make sure that do not puncture the skin of the mouse because it may lead to serious injury or injection-related infections. After pulling back the plunger if there is no blood then the injection is correctly placed. After injecting the animal should be placed at it proper placed.



Figure 7: Subcutaneous Injection in Loose Skin

Intracranial injection should be administered to mice in order to treat the brain tumor. The intracranial injection is injected through the post glenoid because this is most easy pathway for the entrance and the florescent technique is used to locate the tumor. This is most easy procedure for intracranial injection. Usually intradermal needle is used for this purpose. ([Ledwith et al., 2000](#))

Intra thoracic injection can be mainly used to treat and locate lung cancer. First of all the mice is slightly bent and special kind of needle is used that is slightly curved and the injection is placed into the ribcage between two ribs and mainly in the middle and the most important thing to be considered is that it should not puncture the lung .

In case of intra nasal injection the mice are put in cage with face and toe in upward direction that is usually a supine position. It is administered with help of micropipette and injection is usually placed through this. But the main disadvantage is that mice may die due to suffocation. ([H. Y. Wu, Nahm, Guo, Russell, & Briles, 1997](#))

Precautions

Certain points needed to be considered while injecting the most important is that there should be a proper restrainer in which the mouse is placed. Secondly injection may irritations so normal saline can be used to avoid this. All the apparatus being used during the experiment should be clean. Thermometer should be placed in order to check the temperature of the mice. Several precaution should

be taken depending on type of injection e.g., in intravenous injection the vein should be dilated, intramuscular and intracranial injection should be properly placed to avoid the sciatica and tumor respectively. ([Makino et al., 2012](#))

Conclusion

As we know that at present, there are a lot of methods by which blood can be removed from different species of animals in a better way. In the past, blood removal from small rodents was not so easy as there were a lot of hurdles in blood removal. Some methods of blood removal from different animals require specific considerations, i.e., some methods require anaesthesia for smooth removal of blood, and at the same time, there are a lot of side effects when we apply specific methods for blood removal from different animals for any scientific objective, i.e., those side effects may be a pain at the injection site or stiffness of blood vessels. In this modern era, where different scientific technologies and software are available, it is still a huge risk to check the outcomes of a novel drug by directly administering it to humans. Animal testing of drugs should be done by strictly following the animal ethics guidelines. If any injury happens to animals, proper treatment strategies should be applied. As we know, cancer is the leading cause of death worldwide, so those drug bases cancer treatment strategies should be tested on animals to have the best treatment outcomes on humans.

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