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JUGULAR VEIN CANNULATION IN RATS – A MINI REVIEW

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ABSTRACT

Blood is removed from animals for a variety of scientific purposes. As suffering and distress in animals can result in physiological changes which are likely to add another variable to experimental results care should be taken to minimize stress in these laboratory animals as much as possible so that appropriate experimental results could be obtained. Various methods are in use for blood sampling in rodents like tail-clipping, retro-orbital puncture, tail puncture, jugular vein puncture, cardiac puncture, decapitation etc. Vascular cannulations are among the most widely used surgeries in research labs around the world. Cannulation for the repeated blood samples are suitable for use in all strains of rats and can be used to take blood from the femoral artery and vein, carotid artery, jugular vein, vena cava and dorsal aorta. Venous cannulation is said to have advantages which outweigh the arterial cannulation as the former is easier to implant, easier to fill, more likely to maintain patency than the latter. In this respect in addition blood sampling through the jugular catheter offers the advantage that, lost volume can easily be replaced. Thus it is possible to collect sequential samples to evaluate the time course of a response or the acquisition of a larger volume for measurement of hormones which are present at low concentrations in the circulation. Present article is a review with an attempt to provide an elaborate piece of information regarding jugular vein cannulation with precise focus in rats.

Keywords: Cannulation, jugular vein, rats.

INTRODUCTION

Jugular vein and carotid artery cannulations are among the most widely used surgeries in research labs around the world. These cannulations are extremely important for confirmed intravenous delivery of test substances and arterial blood collections. Cannulation reduces the stress of multiple sampling as observed in association with tail vein or orbital sinus technique. Very few adverse effects including a possible rise of corticosteroids and a decrease in platelets are associated with indwelling catheters except under chronic conditions Angela (2007). A cannula (from latin word little reed) is a flexible tube which when inserted into the body is used either to withdraw fluid or insert medication Cannulation (2008). Whereas cannulation is defined as a surgical procedure involving insertion of a flexible catheter into one of the large blood vessels Cannulation (2008). Synonyms used for cannulation are canulation, cannulization, cannulisation, intubation Word Net Search (2008).

Blood vessel cannulation allows repeated blood sampling from conscious, unrestrained rats while avoiding the stress due to handling, restraint and anesthesia (Yoburn *et al*., 1984; Suzuki *et al*., 1997; Reilly, 1998). Surgery may cause changes in hormonal and hemodynamic responses (Weissman, 1990). Catheter infection, while uncommon in rats is known to stimulate a stress response and is a potential consequence of long term catheterization (Popp and Brennan, 1981; Bradfield *et al*., 1992). An adequate recovery time from surgery and aseptic surgical technique may minimize these effects but the precise time for full recovery is uncertain (Fagin, 1983). An adequate recovery time is therefore required to minimize the effects of surgery on a subsequent stressor (Garcia *et al*., 2000). It would be advantageous to use a cannula within days of insertion. Besides the risk of infection in a chronic catheter and the challenge of keeping the cannula patent for more than a week other physiological changes have also been observed (Terao and Shen, 1983).

Blood sampling through the jugular catheter offers the advantage that lost volume can easily be replaced. Thus it is possible to collect sequential samples to evaluate the time course of a response or the acquisition of a larger volume for measurement of hormones at low concentrations in the circulation (Darlington *et al*., 1986; Torner *et al*., 2000) without critically affecting the hemodynamic variables. Chronic cannulation of the jugular vein in freely moving rats and the other larger laboratory animals is an established method for studying drug pharmacokinetics and effects (Popovic *et al*., 1963; Nicolaidis *et al*., 1974; Torres-Molina *et al*., 1992). It allows serial blood sampling with minimal disturbance of the animal without the need for anaesthesia. Commonly used methods for obtaining blood samples such as cardiac puncture or decapitation yield only one blood sample per animal since the animal is killed by the procedure. When

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using techniques like orbital sinus puncture and cutting of the tail serial blood sampling is possible however the sample volume that can be obtained is very small and the procedure is stressful for the animal. Drawing of blood from peripheral sites such as the tail may result in a different pharmacokinetic outcome than using blood taken from central sites (Chiou, 1989[a]; Chiou, 1989[b]).

Need of Cannulation

Frequent sampling increases the stress for the animal and if this is necessary the scientist should consider cannulation. Even in the short term, this technique is a favorable alternative to repeated venepuncture. Cannula (Cann.) can be implanted and used in place of multiple needle entries at any one site, or indeed in place of single sampling from several sites within a relatively short time period.

Short Term and Long Term Cannulation

If sampling over a few hours is required a temporary cann. (short term) such as a butterfly needle or a plastic cann. held in place with tape or some form of bandage could be used. Long term cannulation as indwelling jugular cann. is very suitable for repeated shorterm multiple sampling. Whatever the method employed, surgical skills are essential to position and fix the cann (Gellai and Valtin, 1979; Desjardins, 1986; Dennis *et al*., 1986; Van Dongen *et al*., 1990).

Maintaining Position bf Cannula

In both short term (upto 1 day) and long term (2 days or more) cannulation it may be necessary to restrain the animal in some way to stop it removing the cann. but this depends very much on the species. Smaller mammals like rats are often restrained by some form of harness, swivel and tether along which the cann. runs. Cannulae can be maintained in dogs, pigs, rats and rabbits without the use of harnesses or jackets for example by using simple skin buttons and tygon tubings.

Site of Cannulation

Cannulation is suitable for use in all strains of rats and can be used to take blood from the femoral artery and vein, carotid artery, jugular vein, vena cava and dorsal aorta Blood Sampling Microsite Rat (2007).

Patency of Cannula

In small animals cannulae remain patent for at least 2 to 3 days and some workers have achieved patency for 3 to 4 weeks or longer. Different techniques are available to maintain the patency of the catheter Kohn *et al*. (1997) and Luo *et al.* (2000). Patency can be maintained by using suitable anticoagulant solution as heparin. It is also necessary when considering cannulation to differentiate between infusion and the administration of substances and the removal of blood. It is easier to administer compounds than to remove blood long term, as thrombi attached to the end of a cann. can act as a one way value permitting infusion but restricting withdrawal.

Material of Cannula

Polypropylene polyethylene, nylon and rubber cannulae have been used but silicone rubber or silastic and TygonTM cannulae appears to be the most biocompatible and can be obtained in a sufficient range of sizes. Non-silastic materials tend to cause fibrotic reactions with time whereas silastic types seem to cause little reaction, even after 18 months. The problem with silastic is that it is too flexible arid, therefore predisposed to kinking. It is also easy to obstruct the lumen by over-tightening anchoring ligatures and care should be taken to check patency at the time of operation. To avoid the problem of a small cann. kinking, a polypropylene cann. inside a larger silastic cann. can be used, or a polypropylene cann. can be coated with silastic paint. This will have the effect of combining the greater rigidity of polypropylene with the biocompatibility of silastic. This type of cann. is less easily kinked, easier to flush and will also give accurate pressure measurements than silastic alone.

Placement of Jugular Cannula

Two main mechanisms exist for securing a cann. First by placing it in the lumen of the vein and tying it in place to the vein itself. This is what we have done. Second the cann. can be inserted into a tributary of the target vein with the tip in the lumen of the major vein. The former method involves tying off a vein and destroying its patency but usually this has little effect on the animal. With a jugular cann. the tip may be left very close to the right atrium in the cranial or caudal vena cava. Care should be taken not to place it within the right atrium since cardiac arrhythmias may be induced and this may lead to death by atrial fibrillation.

Sealing the Cannula

The cann. can end in a multiple entry point for example, a silicone rubber stopper can be attached to the end of the cann. and this can be pierced several times. This has the advantage that it is self sealing and will better protect against microbial contamination of the fluid column within the cann. These can be found on a saline drip bag or the plunger seal from a 1 ml disposable syringe and can be inserted into a luer mount of a needle which in turn is inserted into the end of cann. Alternatives are filling the mount with silastic glue, plugging the end with a spigot of solid plastic or metal, heat sealing of the tube ending (Morton *et al*., 1993).

Exit Site of Cannula

Two common sites are there to make the exit of the cann. first at the back of neck and second between the shoulder blades, other exit sites are also possible but over the back is safe as animal cannot interfere with it specially rats cannot chew them in this position usually. Care should be

taken to ensure that cann. should not kink when the animal moves moreover correct length of cann. is very important because if it is too short the cann. may be pulled out or if the length of tubing is too much then it may lead to flexion or kinking. Thus when the rat grows the relative position of the catheter will be shifted which may affect its patency (Thrivikraman *et al*., 2002).

Keeping Cannula Patent after Use

When collecting blood from a cann. the anticoagulant mixture in the cann. should first be removed by drawing it into a syringe until fresh blood appears. The blood sample can then be taken. The "dead space" in the cann. then should be replaced by a fresh anticoagulant to prevent thrombosis. Appropriate anticoagulants include heparin saline (10- 1000 IU/ml) or sodium citrate (0.05% w/v) or either of these in a dense solution such as 25-50% glucose, 5-40% polyvinylpyrrolidine to maintain cann. patency, the cann. should be flushed with saline at least twice a week, if not daily.

Complications with Cannulae

Various complications can occur with cannulae like blockage, infection, accidental removal, gastric ulcers. Rats may develop gastric ulcers because of long term restraint and tethering (Brodie and Hanson, 1966). Appropriate use of anticoagulants & proper flushing of cann. can avoid most of these complications.

Volume of Blood to be Removed

Removal of around 10% of the circulating blood volume will initiate homeostatic cholinergic mechanisms. Cardiac output and blood pressure will be reduced if 15-20% of blood volume is removed. Removal of 30-40% can induce haemorrhagic shock and 40% loss can cause 50% mortality in rats (McGuill and Rowan, 1989). Circulating blood volume in rats is 50-70ml/kg assuming the animal is mature, healthy and on an adequate diet. Circulating blood volume is lower about 15% in obese and older animals. Upto 10% of the circulating blood volume can be taken on a single occasion. For repeat bleeds at shorter intervals a maximum of 1% of an animal's circulating blood volume can be removed every 24 hours.

0.01 X circulating blood volume (ml/day) roughly = 0.6 ml/kg/day.

Care should be taken to replace the blood by infusing equal volume of saline or equal volume of blood from a donor animal. Blood withdrawal and fluid replacement must be performed slowly over 1-2 minutes and at a steady rate. Fluid replacement should be done with sterile, warm physiologic saline equal to the volume of blood collected Mcgill University (2005).

Anaesthesia for Jugular Vein Cannulation

An injectable balanced anaesthetic mixture which is preferred for use is prepared by mixing 1.0ml acepromazine maleate (10mg/ml), 4ml sterile water, 2.5ml ketamine HCl and 2.5ml xylazine HCl in a sealed vial Thrivikraman *et al*. (2000). The older technique of using pentobarbital (nembutol) is also followed. These can be given subcutaneously or intraperitoneally with a 25-G needle. For surgical anaesthesia the mixture is administered at a dose of 125-150µl/100gm body weight and for pentobarbital the dose is 30-50 mg/kg body weight Anesthesia and Analgesia of Mice and Rats (2003). Inhalation (gas) anaesthetics have been used successfully Wotjak *et al*. (1996) and Liebsch *et al.* (1998) for catheterization surgery, however these agents must be used with appropriate gas scavenging systems. Charles River Laboratories in USA are using injectable anesthetics for majority of procedures performed on rodents. Intraperitoneal route of administration is principally used to reduce potential tissue damage from intramuscular and subcutaneous injections. This is accomplished with a one inch needle ranging in size from 20-23gauge. Using a larger gauge needle prevents the inadvertent introduction of anesthetics into the lumen of the abdominal viscera. Smaller gauge needles are more likely to penetrate the lumen of organs due to a high total entry force on the needle tip coupled with the small lumen size of the needle. Typically injections are administered in the lower left or lower right abdominal quadrant with the animal in the head down position. The combination of anesthetics being preferred by them for surgery is Ketamine: Xylazine:sterile water = 2:1:10, administered at the rate of 3.0ml/kg.

Use of Analgesics

The standard default analgesic used by North American Research Models Surgical Services is an opioid, buprenorphine hydrochloride. An alternative non-steroidal anti-inflammatory agent (NSAID), flunixin meglumine is available for substitution. The dosages for Buprenorphine are 0.02mg/kg when under anesthesia and 0.05mg/kg when awake, administered subcutaneously. The dosage for latter is 1.1mg/kg subcutaneously every 12 hours if necessary Surgical Capabilities (2005). The same analgesic was dissolved in Nutella chocolate & provided to rats at a dose of 0.4mg/kg body weight every day, as a means of postoperative pain relief Flecknell (1998).

Heat Loss

Because the ratio of body surface area to mass is greater in small rodents than in large domestic animals, heat dissipation during surgery & post-surgical recovery is common with general anesthesia. This can cause significant variations in the metabolism of anesthetics and hence the rate of recovery. This heat loss also affects cardiovascular performance, as well as the urinary excretion of anesthetics, thereby prolonging anesthesia. For this reason during the surgical period, as well as postoperatively, supplemental heat is provided to the

animals via heated surfaces. The temperature of the heating devices should be closely monitored to avoid harmful elevation in temperature on the skin's surface that could result in burns. Generally the animals are removed from the heated surface when their righting reflexes are regained and they can maintain normal posture Surgical Capabilities (2005).

Surgical Technique

Precautions

A. Surgical gloves, eye protection, long-sleeved gown, closed in shoes.
B. All instruments and materials (including catheter) must be sterilized before use.
C. Surgery must be performed under aseptic conditions

Equipments

A. Anaesthetic
B. 70% Alcohol
C. Heparinized saline
D. Scalpel (No 22), scissors, forceps, haemostats
E. Silk ligatures
F. Suture material or staples
G. Sterile Cannula (Cann) O.D 1.05mm x I.D 0.5mm

Procedure

1. Anaesthetize rat (specify dose, route and volume).
2. Lay the rat on its back with the head away from the surgeon.
3. Measure the distance required by the cann. (around 3-4cms or ~20 cm if exteriorizing via stainless steel spring at the back of neck).
4. Shave hair on the ventral neck from midline to 1cm past jugular groove and swab the skin with alcohol.
5. Make a 1.5-2.0mm incision in the neck to one side of the midline. Blunt dissect away the fat and connective tissue.
6. Pass a pair of haemostats under the vein and place 2 ligatures around the vein.
7. Using blunt dissection, clear the area of connective tissue and fat until the bifurcation of the internal and external jugular veins is exposed.
8. Loosely position ties around the internal and external branches and the common jugular vein (i.e., one tie on each of the three branches of vein).
9. Raise the posterior ligatures and hemi-transect the vein between the two ligatures.
10. Introduce a heparinized saline filled cann. into the vein and advance it towards the heart. Verify the patency of the catheter by withdrawing blood. Flush with heparinized saline.
11. Tie the anterior ligature firmly around the catheter once inside the common jugular vein. Tie the posterior ligatures around the cann. and vein.
12. Grasp the cann. and create a "stress loop"
13. Secure the catheter loop with the posterior ligatures
14. Shave the dorsal neck and swab with alcohol.
15. Make a 0.5-1.0ml incision and create a s/c tunnel using a straight pair of haemostats.
16. Cut the cann. leaving 2.5-3.0cm exterior to the skin.
17. Suture the skin or pass the catheter through a stainless steel spring and suture the base of the spring into the subcutaneous pocket at the back of the neck The University of Queensland (2004).

In fact it would not have been possible to evaluate temporal pattern of hypothalamic pituitary adrenal (HPA) axis responses to stressors in conscious rats without the use of repeated blood sampling technique Plotsky *et al*. (1992) Engelmann *et al.* (1996), Thrivikraman *et al*. (1997), Thrivikraman *et al*. (1999), Ladd *et al.* (2000), Arborelius *et al*. (2000) and Huot *et al*. (2001). Vascular access techniques have been employed to collect blood samples since 1960's Cocchetto and Bjornsson (1983) and Lestage *et al.* (1985) in many neuroendocrinological Pich *et al*. (1993), Bohus (1998) and Lightman *et al*. (2000) and pharmacological studies Rawlings *et al*. (1994), Booze *et al*. (1997) and Rivier *et al.* (1999).

As most of our present knowledge in animal physiology comes from experiments performed on anesthetized animals or from in vitro studies the utility of these preparations is often curtailed when highly complex neural or hormonal regulatory mechanisms are investigated. Basal secretion rates and plasma levels of most hormones are drastically influenced by anesthesia and surgical trauma (Pettinger, 1975; Depocas and Behrens, 1977; Carruba *et al*., 1981) and even simple handling of animals may have profound effects. Thus from an ethical perspective, there is a moral mandate to strive to reduce stress in laboratory animals during normal husbandry as well as during and after experimental procedures. From a scientific point of view, stress is a well known and unwanted source of experimental error, because the natural response of an animal to stressors includes alterations in the normal physiology and metabolism. This may add between animal variation in responses to experimental procedures Hau *et al*. (2001) and Morton and Hau (2002).

In conclusion stress in animals is a recognized cause of experimental errors. Use of vascular access in small laboratory animals is a delicate and very good tool for the scientists and researchers to avoid stress and carry out the time course studies. In addition it is helpful in determining the cytokine and hormonal levels using less number of animals at a time. Jugular vein cannulation being a surgical procedure, is also associated with disadvantages but these are few and minor like it requires skill and time consuming, it is not very easy in comparison to tail puncture or tail clipping and if jugular vein happens to be ruptured it can be dangerous for the animal but all these can be easily overcome by carefully practicing the surgical skill. Thus Jugular vein

cannulation is a better method of cannulation in comparison to other vascular access ports in rats as it provides the overwhelming advantage of replacing the lost volume in the animal besides giving an opportunity to get larger blood volume for measurement of hormones which are present in circulation in low concentrations.

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