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Original article

EXPERIMENTAL SURGICAL PROTOCOL FOR CONTINUOUS FEMORAL VENOUS ACCESS IN THE RAT

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Summary

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Access to the circulatory system is essential for continuous monitoring and long-term experimental study. One of the commonest methods for venous access is femoral vein cannulation. The aim of the presented protocol was to elaborate a surgical model for cannulation of femoral vein in rat. Temporary cannulae such as butterfly needles can be used in short-term procedures, whereas long-term monitoring required surgical implantation of a biocompatible cannula. The protocol allows direct venous access, continuous monitoring and evaluation of the tested compounds in experimental pharmacology and toxicology – the tested compounds reach the systemic circulation immediately and the dose can be accurately titrated against response. The protocol can be applied as an alternative for venous access in experimental clinical settings.

Key words: experimental surgery, femoral cannulation, venous access in rats

INTRODUCTION

Rats have long been recognised as a valuable biomedical research model, notably in the investigation of aging, toxicology, addiction, and common human diseases such as diabetes and hypertension. In many instances, individuals conducting such research studies are charged with important responsibilities, including animal facility management, animal husbandry, veterinary care, regulatory compliance, and various experimental methodologies. With the advent of genetic manipulations and biomedical research technological advances such as bioimaging, the versatility and usefulness of the rat as an animal model has increased (Suckow, 2006).

Long-term monitoring and prolonged experimental study require access to the circulatory system. All routes of central

venous access are associated with complications and possible failure. The predictable anatomic locations of the internal jugular, subclavian, and femoral veins make them easy for cannulation. One of the commonest method for venous access is via femoral vein cannulation. The intravenous route permits infusion of fluids and drugs and allows immediate access of the administered dose of tested compounds to systemic circulation. Arterial and vein catheterisation have historically been used for both acutely and chronically monitor blood pressure, blood sample collection and substance delivering in experimental rat animal models. Catheterisation of the femoral vein is used to perform injections or continuous infusions of a substance. On the other hand, catheterisation of the femoral artery is used to collect multiple blood samples in volumes higher than 100 µL or to measure the arterial pressure (Di Loreto & Rigalli, 2009). Rats are commonly used in the laboratory for a multitude of scientific studies due to their small size and convenient handling. There are several locations where a chronic catheter can be placed within an animal, including the jugular vein, abdominal aorta, carotid artery and femoral artery, to name a few. Cannulation of the femoral vein can be used for infusion of toxins and drugs and repeated blood sampling over prolonged periods. Therefore, vascular access in a small animal model is critical for studying clinical performance measures and treatment effects (Feng *et al.*, 2015). A temporary cannula e.g. a butterfly needle can be used in the short term (working day), whereas for long-term use surgical implantation of a biocompatible cannula is required. This method allows repeated blood sampling with minimal distress and discomfort for the animal (Diehl *et al.*, 2001). For more prolonged infusions, administration of test substance via the femoral vein catheter may be preferable as its patency is easier to maintain similar to that in the jugular vein but the risk of damage to the heart from the catheter is avoided (Gad, 2016).

The main goal of this study was to elaborate a surgical model for small laboratory animals, implemented in a preliminary study of biological and chemical compounds with toxic effects and potential antidote treatment. For the purpose of the current study cannulation of femoral vein in rats was performed. This provides opportunities for pharmacokinetic and toxicokinetic studies, and prolonged monitoring of the tested compounds.

MATERIALS AND METHODS

Drugs

The following substances were used: saline 0.9% NaCl (Actavis, Bulgaria); heparin natrium 250 IU/mL (Braun); 20% urethan in d.H_2O (Fluka), prepared *ex tempore*; lidocaine 1% (Sopharma, Bulgaria); povidone-iodine 10% (Chemax Pharma).

Microsurgical sets

Used microsurgical sets comprised: thermostated rat board (World Precision Instruments, Inc., Sarasota, USA); Vessel Cannulation kit for laboratory animals – scalpels, hemostatic forceps, tissue forceps, colibri forceps, curved, debakey forceps, surgical needles, needle holders, microvascular clamps, tenotomy scissors, adventitia scissors, vannas scissors, tubal dissecting scissors, retractor, (World Precision Instruments, Inc., Sarasota, USA); Vessel catheter Portex ID 0.58 mm/ OD 0.96 mm; Surgical binocular loupes 504038-2.5× (World Precision Instruments, Inc., Sarasota, USA).

Laboratory animals

Experiments were carried out on 20 male albino Wistar rats (180–220 g) obtained from the Animal Farm of the Military Medical Academy. Prior to the experiments they were housed with 6 animals per cage. Temperature was kept at 18–22°C, humidity was maintained at 50–65% and 12 h light-dark cycle was available. Rats were fed standard rodent food and allowed *ad libitum* access to tap water. Experiments were carried out in accordance with the requirements for the protection and humane treatment of experimental animals (Anonymous, 2012).

The scientific, experimental *in vivo* examination of experimental animals, has been conducted in accordance with the principles of ICLAS/FELASA and Ordinance 20/2012, with regard to which the Military Medical Academy has received the necessary license (license 13/06.02. 2013/ Standpoint № 6 / from the National Commission expiration date: 06.02.2018).

RESULTS

The surgical protocol comprised the following stages:

Preparation of the laboratory animal: 48 h before the experiments all laboratory animals were given free access to food and water, each animal lived separately in cage. At least 4 h before the anaesthetic period food and water were withdrawn.

Anaesthesia: Appropriate *i. p.* anaesthesia using 1.5 g/kg 20% Urethan was applied 45 min before the beginning of experiment to minimise any pain and discomfort (Fig. 1).

Preparation for the surgical procedure (preoperative phase): The anaesthetised rat was placed in dorsal position and the legs were restrained to each side of the thermostated table (Fig. 2).

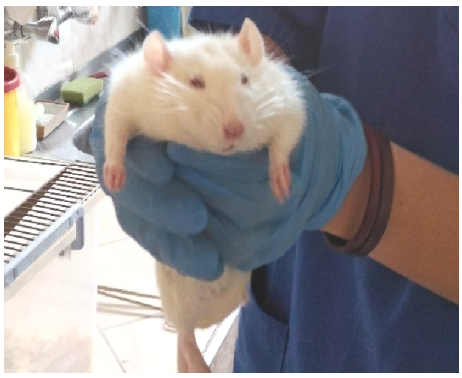


Fig. 1. Preparation for anaesthesia.

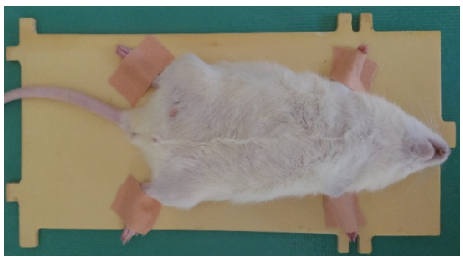


Fig. 2. Preparation for the surgical procedure.

Surgical procedure steps (intraoperative stage) are described on Fig. 3. The femoral arterial pulsation of the hind leg was identified (Fig. 3-1). The puncture point of the femoral vein was approximately 1 cm below the inguinal ligament (Fig. 3-2). For percutaneous access to the femoral vein, an incision in the inguinal area was made, the connective tissue under the skin was dissected to expose the femoral vein (Fig. 3-3). The vein was ligated and located under the gracilis muscle (Fig. 3-4). After the femoral vein was localised, artery-vein separation was necessary (Fig. 3-5). In order to avoid blood vessel retraction due to mechanical stimulation, 2–3 mL 1% lidocaine was injected 2–3 min prior to dissection of the adventitia and its dissection from the artery and femoral nerve (usually they are positioned dorsomedially; the artery with a smaller diameter than the vein) (Fig. 3-6).

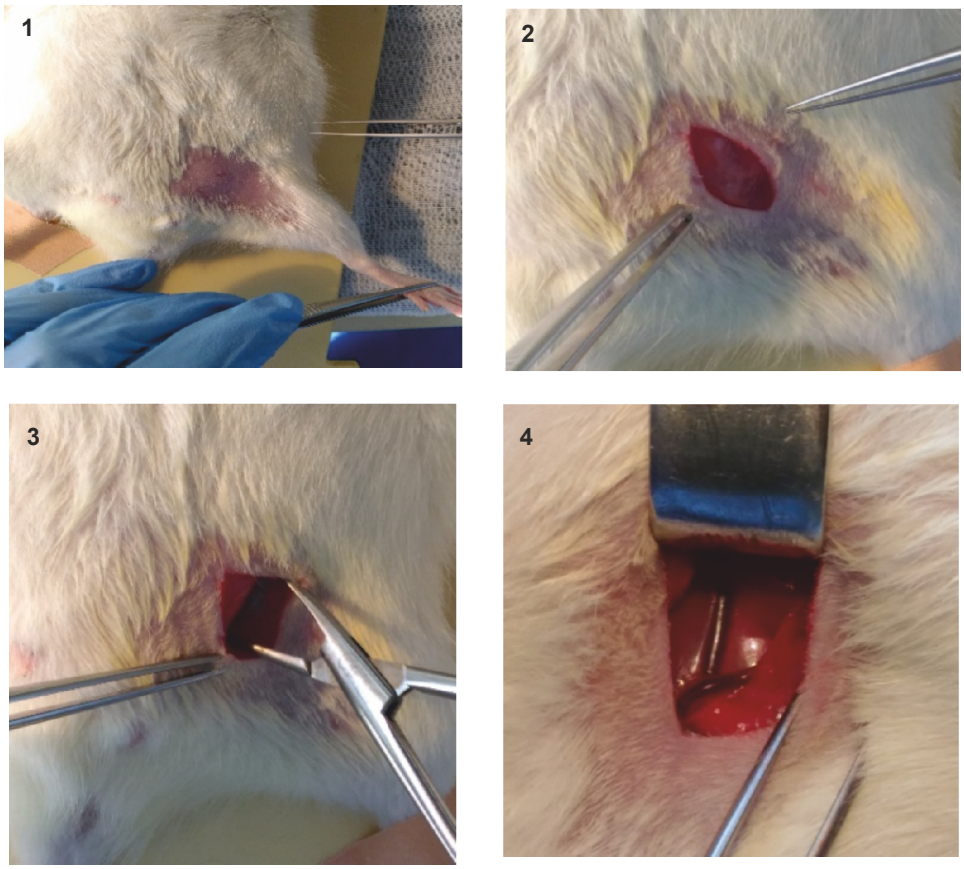


Fig. 3. Surgical procedure steps: identification of the femoral arterial pulsation and puncture point (1), incision (2), dissection of the connective tissue (3) and femoral vein identification (4).

Once the femoral vein was separated, a loose ligature was tied on the distal and proximal part of the vessel (Fig. 3-7). Using micro-dissecting scissors, the vein was incised under a 45-degree angle. The venous catheter was placed into the vein (Fig. 3-8). When the catheter was fully inserted, the ligature was tightened around the vein and catheter, and a triple knot was tied (Fig. 3-9).

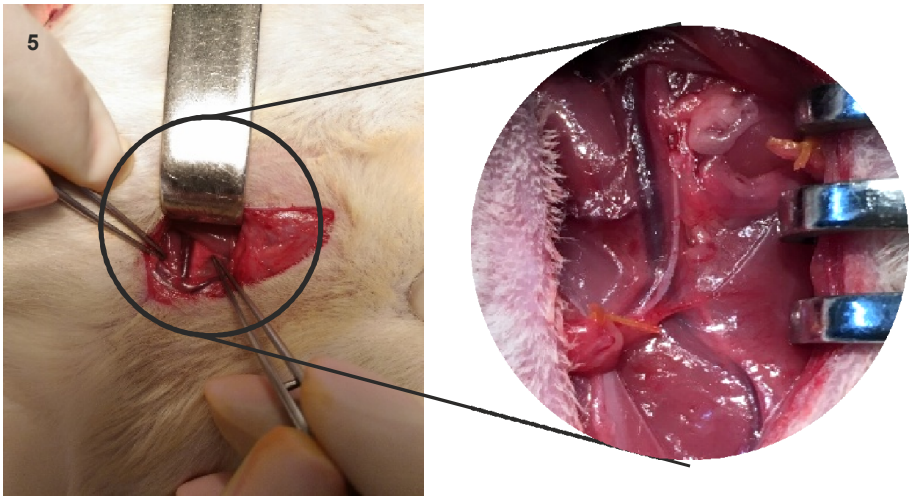
Cannulation function was verified by the presence of blood in the catheter. The catheter had a small diameter, hence the risk from blood clotting. To prevent this flushing with an anticoagulant (250 IU/mL heparin) was required.

A three-way stopcock can be used if multiple injections are required (Fig. 4).

DISCUSSION

The experimental surgical model described above allows a long-term venous access and prolonged experimental studies to be carried out in rat models, which are widely used in experimental toxicology

and pharmacology. Under these conditions the influence of different drugs or toxic agents could be investigated and assessed. The femoral vein as well as other vessels (jugular vein, vena cava and tail vein) are most commonly used for catheterisation and acute or chronic continuous infusion of test substances (Koch, 2006). The surgical method described above is applicable, for example, in case of evaluation and assessment of the toxic effects of some natural or synthetic poisons on the neuromuscular transmission (NMT). Neuromuscular junction is a target place for the effects of nerve agents resulting in neuromuscular block (NMB) and paralysis of skeletal muscle. When NMB occurred, the antidotal effect, e.g. restoration of NMT of some drugs could be assessed. Such type of experiments require intravenous administration of both toxic agent and antidotes and observation within the framework of 60–120 min. The traditional routes of application of poisons and drugs (*i.m.* or *i.p.*), are not suitable in this case.



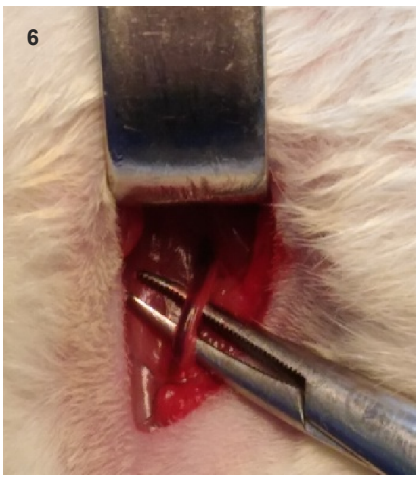


Fig. 3. Surgical procedure steps: artery-vein separation (5–6).

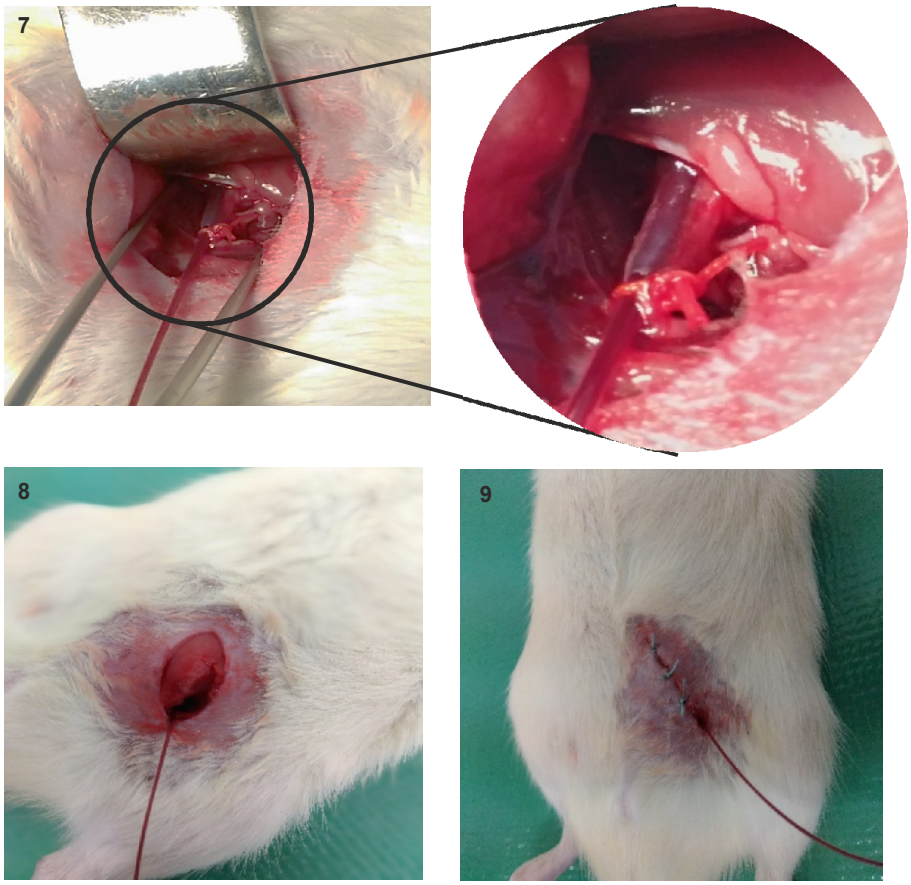


Fig. 3. Surgical procedure steps: ligation of the distal and proximal part of the vessel (7), catheterisation of the femoral vein (8–9).

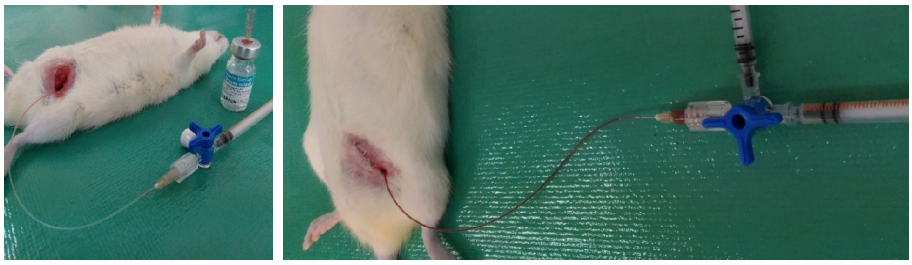


Fig. 4. Heparinisation to prevent clotting.

There are multiple protocols that are utilised for drug administration and investigation, including tail cuff techniques, jugular and subclavian vein cannulation. Tail cuff methodologies are less accurate, they do not require surgery and can also

be repeated, but do not permit easy simultaneous blood sampling or drug administration. Even with a properly validated tail-cuff method, errors can still occur particularly when it is used to quantify modest blood pressure changes, or following administration of vasoactive drugs (Buñag, 1983).

The rat femoral site for chronic catheterisation results in increased length of catheter patency and is characterised with the fastest recovery of pre-surgical animal weight compared to other catheter locations (Jespersen *et al.*, 2012). As patency is concerned, the femoral implantation tended to remain patent longer than the jugular one. Thus considering both patency and surgical stress the femoral vessel procedure is the most desirable (Yoburn *et al.*, 1984). One advantage of this route is that the vein is large and allows easy access distant from the vital intrathoracic structures, its distance from the head and heart presumes minimum interference with the evaluation and treatment compared to other locations where a chronical catheter can be placed – jugular vein and subclavian vein (Wald & Coté, 2009).

There are several disadvantages to this method: it is a surgically invasive technique, requiring competence and specific technical skills to perform venous cannulation, damage to the vein due to the implantation of the catheter and the potential of infection.

The following possible errors and complications should be noted. The needle for the catheter could penetrate through the whole vessel, which is solved by distal ligation of the vessel and proximal recannulation. A "deep" positioning of the catheter could occur – with its transition from the femoral into the iliac vein. This issue is solved by pulling out the catheter and recannulation. Unsuccessful fixation of the catheter could be due to incomplete dissection of the vessel. The errors described above had occurred in 4 of 20 rats due to insufficient technical skills in the beginning of experiments rather than disadvantage of the surgical method discussed in the current article.

In conclusion, a protocol for cannulation of *v. femoralis* has been developed permitting long-term monitoring and conducting a prolonged experimental study. Accordingly, it is feasible to use intravenous route of administration to be sure that the entire administered dose of any tested compound will reach the systemic circulation immediately. The technique could be applied for assessment of toxic effects of poisons from different origin and their antidotal treatment. This experimental model could be successfully applied in the process of education of undergraduate or post graduate students and researchers.

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