Simple Reliable Method for Chronic Cannulation of the Jugular Vein for Pharmacokinetic Studies in Rats

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Received December 3, 1981, from the *Department of Pharmacodynamics, College of Pharmacy, University of Illinois Health Sciences Center, Chicago, IL 60612.* Accepted for publication March 5, 1982.

Abstract □ A simple method for the preparation and implantation of silicone cannulas into the rat jugular vein is described. The implanted cannula can be used to administer drugs and collect blood samples at intervals of ≤1 min without causing stress to the animal. If necessary, the animals can be exanguinated within a few minutes using this cannula. With proper maintenance, the cannula is patent for weeks and could be used for repeated and crossover studies.

Keyphrases □ Sampling, blood—serial, chronic cannulation procedure, rat jugular vein, pharmacokinetic studies □ Cannulation—chronic, rat jugular vein, serial blood samples for pharmacokinetic studies □ Pharmacokinetics—serial blood sampling by chronic cannulation, rat jugular vein

Pharmacokinetic studies of drugs require sequential blood sampling. It is preferable that the blood samples are drawn from the animals with minimum trauma and stress to avoid any changes in the body physiology, which might alter the disposition characteristics of the drug (1–4). A number of methods are available for serial blood sampling (5, 6); these can be broadly classified into surgical and nonsurgical methods. The nonsurgical methods include cardiac puncture (5, 7), bleeding of the orbital plexus (8–10), and bleeding of the tail (5, 11). These methods are traumatic and may require anesthetizing the animals; the cardiac puncture method is not suitable for sequential blood sampling. Some methods do not involve surgery and are available for continuous intravenous drug infusion (12–14), but these methods cannot be used for serial blood sampling because the vein often collapses after a certain period of time.

The surgical approaches for obtaining serial blood samples mainly include cannulation of the jugular vein (15–19) or cardiac ventricle (20, 21). Other methods of cannulation in the rat for the study of first-pass effect have been reviewed elsewhere (6).

The cannulation of the external jugular vein is one of the most reliable methods for intravenous administration of drugs and rapid sequential blood sampling for prolonged periods of time. However, the complexity involved in the cannulation and maintenance of cannula patency for a sufficient length of time has limited the use of this technique in pharmacokinetic studies. The purpose of this study is to devise a simple method for the preparation of the cannula, for the introduction of the cannula into the vein, and for the maintenance of cannula patency for long-term use.

EXPERIMENTAL

Preparation of Cannula—Approximately 12 cm of medical-grade silicone rubber tubing (0.051-cm i.d. × 0.094-cm o.d.)[1] was gently wrapped around twice with silk thread[2] ~20 cm in length. A firm knot was tied in the thread, taking precaution that the lumen of the silicone tube was not occluded. The knot was made ~3 cm from one end of the silicone tubing, so that the other end was ~9 cm long (Fig. 1a).

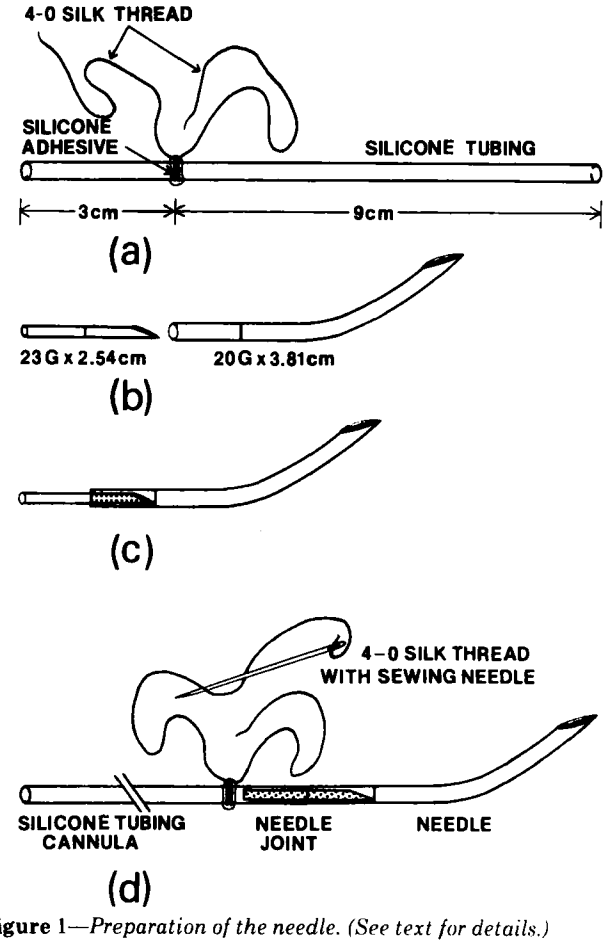

Figure 1—*Preparation of the needle. (See text for details.)*

A coat of silicone adhesive[3] was applied to the thread around the tubing and cured for at least 24 hr. The coating was not >1 mm thick so as to keep the thread firmly held on the silicone tubing without making the joint too bulky. After curing, the silicone cannula was checked for occlusion by flushing with sterile normal saline. The cannulas were cleaned and disinfected by soaking them in benzalkonium chloride antirust solution for at least 30 min before use. The lumen of the cannula was cleaned by flushing with 2 ml of sterile normal saline.

The short end of the cannula is inserted into the superior vena cava through the right external jugular vein, and the longer portion is passed subcutaneously to the back of the neck. The length of the short end can vary depending on the size of the rat: a 3-cm length is suitable for rats weighing between 300 and 450 g.

Preparation of the Implantation Needle—The needle used for the introduction of the cannula into the jugular vein was prepared using the method of Harms and Ojeda (17). The plastic hubs were removed from two thin-walled beveled hypodermic needles[4] (23-gauge × 2.54 cm and 20-gauge × 3.81 cm). Approximately 1.2 cm of the beveled end of the

[1] Silastic Medical-Grade Tubing, Dow Corning Corp., Medical Products, Midland, Mich.
[2] 4-0 Silk, Black Braided, Code A-53, Ethicon, Inc., Somerville, N.J.
[3] Silastic Medical Adhesive, Silicone Type A, Dow Corning Corp., Medical Products, Midland, Mich.
[4] Yale Sterile Disposable Needles, Becton, Dickinson and Co., Rutherford, N.J.

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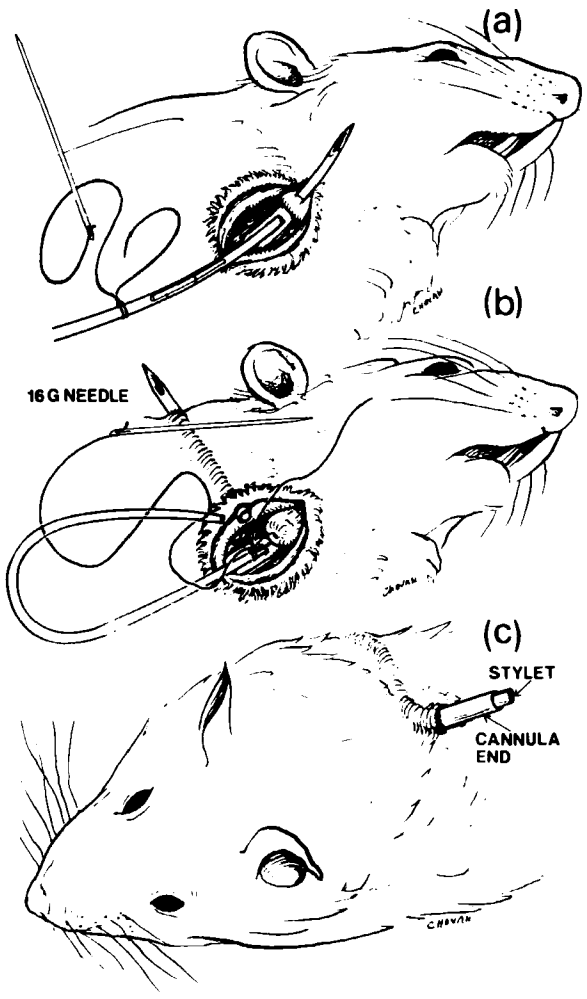

Figure 2—*Cannulation of the rat. Key: (a) insertion of the needle, (b) securing of the cannula, and (c) externalization of the cannula.*

23-gauge needle was forced into the opposite (nonbeveled) end of the 20-gauge needle, which had been bent (Fig. 1b). If the 23-gauge needle was too thick to be fitted snugly into the bore of the 20-gauge needle, the outer surface of the 23-gauge needle was rubbed on a rough surface (such as a sharpening stone) to make the outer diameter small enough to fit tightly into the lumen of the 20-gauge needle. The short end of the silicone cannula fit snugly over the protruding portion of the 23-gauge needle (Fig. 1c).

Surgical Implantation of the Cannula—All the instruments used for the animal surgery were cleaned and disinfected by immersing them into a benzalkonium chloride antirust solution for ~1 hr. Strict asepsis was not necessary, but a clean environment and neat handling of the animal were important.

The rat was anesthetized with ether in a desiccator. After removing the rat from the desiccator, the anesthesia was maintained throughout the implantation procedure by placing ether-impregnated cotton close to the nose of the rat. The hair on the back of the animal was clipped, and a point was marked at the center of the back of the neck to indicate the exteriorization point for the longer portion of the cannula. The rat was then placed on its back, and the hair on the area over the right external jugular vein was clipped. This area is recognized by observing the rapid pulsation of the jugular vein. The shaved area was cleaned by repeated wipings with sterile gauze saturated with 70% isopropyl alcohol.

A longitudinal incision, ~2 cm long, was made on the skin over the jugular vein, and the vein was exposed by clearing the surrounding tissues. To avoid unnecessary blood loss, care was taken not to damage any surrounding veins. The short end of the cannula was passed into and out of the jugular vein using the implantation needle–cannula assembly (Fig. 2a). This was done without loss of blood from the vein.

At this point, while the shorter end of the cannula was out of the jugular vein, a sterile disposable syringe (3- to 10-ml capacity) containing heparinized (20 U/ml) normal saline for injuection, was connected to the longer end of the silicone cannula by a blunt-ended 23-gauge sterile needle. This needle was prepared by clipping the beveled portion of a 23-gauge × 2.54-cm needle, smoothing out both the inside and outside of this cut-end needle, cleaning and sterilizing with benzalkonium chloride antirust solution, and rinsing several times with heparinized normal saline for injection. The flushing can be done with or without the implantation needle at the end of the cannula. However, it is a good practice to flush the initial portion of the normal saline with the implantation needle on, so that the needle is cleared of any blood that may have entered it during its short passage through the jugular vein. If the implantation needle is not flushed immediately, blood clots may form in the lumen of the needle; these clots often are difficult to remove.

The implantation needle was disconnected and the cannula was flushed again with ~0.5 ml of normal saline. Then the cannula was pushed slowly toward the heart until the junction of the silk thread was reached. The cannula was then fixed in place by suturing the silk thread to the muscle through which the implantation needle exited (Fig. 2b). The suturing was done using an ordinary sewing needle which had been sterilized.

Approximately 0.2 ml of heparinized normal saline was injected slowly from the syringe through the cannula into the vena cava. The plunger of the syringe was then pulled back slowly to observe the appearance of blood in the cannula. When blood was observed, another 0.2 ml of heparinized sterile normal saline was injected into the cannula. The longer end of the cannula was disconnected from the syringe and threaded with the aid of a 16-gauge needle (Fig. 2b), which was passed subcutaneously from the point marked at the back of the rat's neck to the point of incision. Blood loss through the cannula was minimized during this step either by putting a small clip close to the junction of the silk thread or by keeping the cannula pressed at that point with two fingers. After passing through the skin, the cannula was again filled with heparinized normal saline and closed with a 1-cm portion of nylon monofilament, the inner end of which had been tapered and smoothed.

The muscles at the incision were closed by a single suture, and the open skin was closed by 3 to 4 stitches using absorbable, surgical suture with a needle[5] and hemostat. The administration of ether to the rat was then discontinued, any blood around the skin incision was cleared with cotton gauze, and the rat was placed in a supine position in its cage. Within a few minutes, the rat turned over and started walking somewhat shakily. After 1 hr, the movements of the rat became normal, indicating that the rat had recovered from the anesthesia.

Serial Blood Collection—To avoid contamination of blood from one sample to the next, a 1- or 3-ml disposable plastic syringe was fitted with a 23-gauge × 2.54-cm needle (Fig. 3). The beveled portion of the needle had been cut off and smoothed, as mentioned earlier, to avoid damaging the cannula. The needle was fitted with two segments (A and B) of silicone rubber tubing, both ends of which had been smoothed (Fig. 3). At the end of the second tubing (B), another 23-gauge connector was fixed with half of its length protruding.

When blood was collected, the nylon stylet was removed from the implanted cannula, and the cannula was connected to the free end of the 23-gauge needle. When the plunger of the syringe was pulled back, first heparinized normal saline and then blood started entering the tubing and the syringe. As soon as the dead volume of normal saline and/or blood had crossed the joint of the two tubings, the first tubing (A) was disconnected along with its needle joint. Another syringe, fitted with a 23-gauge blunt-ended needle, was attached to the open end of the second tubing (B). Blood was drawn into the second tubing by pulling the plunger back slowly.

During the connecting and disconnecting processes, the open end of the cannula was firmly pressed between two fingers so that the blood remained immobile within the cannula. After drawing the required volume of blood, the syringe and its needle were removed, and the cannula was filled with heparinized normal saline and closed with the nylon stylet. If blood samples are withdrawn in quick succession, for example every minute, flushing the cannula each time with saline solution is unneces-

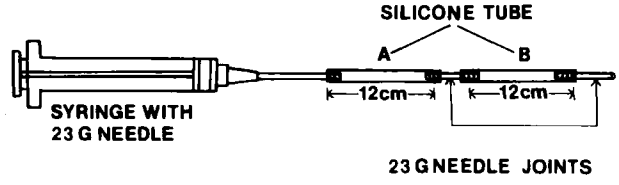

Figure 3—*Syringe and tubing assembly for the collection of uncontaminated serial blood samples from cannulated rats.*

[5] 4-0 Dexon, American Cyanamid Co., Pearl River, N.Y.

sary. The mixing of blood between samples can be avoided by adopting the above procedure.

Maintenance of the Cannula—Using the syringe and tubing arrangement, as described earlier for the collection of serial blood samples, the blood was first drawn into the tubing under negative pressure. If any clot had formed, it was removed from the cannula and not pushed into the systemic circulation. The cannula was then filled completely with heparinized (20 U/ml) normal saline for injection. Flushing of the cannula was done at least once, but preferably twice, a day.

DISCUSSION

The method described here for the chronic cannulation of the rat jugular vein is simple and reliable. More than 100 rats have been cannulated in our laboratory for pharmacokinetic studies of drugs whose half-lives ranged from a few minutes to several hours or days. With proper maintenance, the cannula remains patent for months. This method for the preparation of the cannula is simpler than the one described previously (17). The cannula preparation eliminates the use of silicone sheeting which is difficult to fabricate, causes discomfort to the rats, has a bulky appearance after the skin incision is closed and healed, and prolongs the healing time. Other methods of cannula preparation (16, 18) are even more complicated, needing many accessories and expertise to make and implant them.

Upton (19) described a cannulation method where the "tubing is secured by tying firmly around the vein," which blocks blood circulation through the jugular vein. The cannula can become blocked if tied too firmly, or dislodged from the vein if the knot is not firm enough. Insertion of the cannula through the jugular vein into the vena cava involves manipulation of the vein which can cause it to collapse, thus complicating further the insertion procedure. Unless the tube is inserted promptly on incision in the jugular vein (19), significant loss of venous blood may occur. However, the method described here involves minimal manipulation of the vein with little loss of blood.

The passage of the cannula into and out of the jugular vein should be accomplished in the first attempt. If the first attempt fails, the vein may collapse, making it somewhat difficult (but not impossible) to insert the implantation needle into the vein. It is recommended that while learning this cannulation technique, one should anesthetize the first few rats with pentobarbital, since ether anesthesia requires attention to maintain the optimum anesthesia of the rat, which may hamper the surgical procedure.

Monofilament nylon cord is used as a stylet to close the open end of the cannula because it is nonreactive with normal saline and the silicone tubing and is lighter than stainless steel. Although the medical-grade silicone tubing used is nonreactive with body tissue (22), it neither resists blood clotting nor is impervious to moisture. Thus, loss of water from the saline-filled cannula allows the blood to enter the cannula and form a clot. Initially, this clot is soft and could be aspirated easily. But if the clot remains for a longer period (>24 hr), it becomes hardened and strongly affixed to the inside wall of the cannula. In these cases, heparinized normal saline should be forced in to clear the cannula. The presence of these clots in the vena cava often damages this blood vessel, rendering the cannula inoperative. It is preferable that the clots be removed by aspiration before flushing the cannula rather than be forced into the general circulation. To reduce the microbial contamination during the flushing of the cannula, the exposed end of the cannula, the stylet, the needle of the syringe, and the finger tips which come in contact with the cannula should be thoroughly wiped with sterile gauze saturated with 70% isopropyl alcohol.

For the first few days, some rats may show some restlessness making it difficult to connect or disconnect the syringe with cannula for drawing the blood. In these situations, some confinement such as that described by Davis and Coleman (23) or any simpler version, should be used to limit the movements of the animal. After time the animal becomes used to this manipulation and offers no problems.

Unless the pharmacokinetic studies are complicated by the use of heparin (24), it is recommended that blood sample volumes should be replaced by heparinized (20–50 U/ml) normal saline. Also ~0.2 ml of heparinized (20–50 U/ml) normal saline should be injected a few minutes before the withdrawal of blood. If heparin poses problems, another anticoagulant may be used.

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