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TITLE

A CHRONIC ARTERIAL CANNULA FOR BLOOD SAMPLING IN CONSCIOUS, UNRESTRAINED RATS

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A Chronic Arterial Cannula for Blood Sampling in Conscious, Unrestrained Rats

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Abstract | We require repeated blood samples for at least 10 days from conscious, unrestrained rats in our studies investigating the inflammatory response to intra-abdominal infection. Furthermore, we require a means by which other experimental devices (implanted concurrently) can be kept out of reach of the animals for the duration of the study. Under these conditions, previously reported postoperative restraint and cannulation techniques did not allow cannula patency for longer than a few days. We designed a cannula that is constructed from microbore tubing, attached to a modified winged infusion catheter, and sutured over the scapulae of the animal. Our device features a curved, perforated tip to allow its easy insertion into the thoracic aorta via the left carotid artery, and it provides an attachment site for a small, lightweight backpack. This cannula has a small dead-space and accommodates repeated arterial blood sampling for at least 10 days without leading to bacteremia or significant alterations in hematological or immune parameters. With minor modifications, this cannula has potential application in the chronic cannulation of other vessels and ducts in other small animals.

In our studies investigating the inflammatory response to intra-abdominal infection, we require repeated blood samples from conscious, unrestrained rats. Furthermore, our protocol requires a means of keeping other devices that are implanted (e.g., peritoneal cannula and thermocouple) concurrently with catheterization out of the animals' reach for at least 10 days. Under our experimental conditions, conventional postoperative restraint techniques (e.g., fitting animal with an Elizabethan collar or a nylon mesh harness and tether) did not maintain cannula patency and/or ensure device integrity for longer than a few days. Although several investigators have successfully cannulated the rat tail vein for chronic blood sampling (1, 2), these methods were unsuitable for our purposes because we could not simultaneously shield our other experimental devices from the animals.

Several blood sampling devices and surgical implantation procedures have been described to chronically cannulate some of the larger internal blood vessels in conscious, unrestrained rats. Cannulae have been implanted in the aortic arch via the left or right carotid arteries (3, 4); in the abdominal aorta via the femoral artery (5); in the superior vena cava via the left external jugular vein (5–7); in the inferior vena cava via the femoral vein (8); and in the heart via the right external jugular vein (9–11). Various types of cannulae have also been introduced at different depths in the external jugular veins (12–16). Investigators have claimed that their cannulae were patent from periods ranging from a few weeks (3) to 1 year (10). However, it has been our experience that many of these methods did not allow blood withdrawal in several experimental animals after 3–5 days due to formation of an extensive fibrin sleeve around the cannulated blood vessel. This inflammatory response has also been reported after implantation of cannulae in other models (17, 18) and was not reduced when we performed cannulation under strict aseptic conditions. Furthermore, we could not use previously reported methods of cannulation because they involve major abdominal surgery (5, 17–19), which may considerably alter the immunological response to infection (20, 21).

Here we describe a simple, easily constructed cannula that can be used for repeated blood sampling from the thoracic aorta for at least 10 days as well as for delivery of fluids in freely moving rats. One innovation of our cannula is that it also provides an attachment site for an optional backpack for storing other lightweight devices required by the experimental protocol. Data is presented showing that this method does not significantly alter hematological or immune parameters.

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Materials and Methods

Animals. We obtained 24 specific-pathogen-free, male Sprague-Dawley rats (*Rattus norvegicus albinus*; mean body weight ± SEM, 384 ± 15 g) from Charles River Laboratories (St-Constant, QC, Canada). The animals were housed individually in polycarbonate boxes and allowed to adapt to the environmental conditions (21°C, 12 h light/dark cycle) for 7 days. To reduce the changes in stress hormones during the experimental period, rats were handled daily for 7 days prior to surgery (3, 22). All animals had free access to commercial rodent chow (B-W Feed & Seeds Ltd., New Hamburg, ON, Canada) and water at all times during the experimental period. All procedures described in this study were performed in adherence to the Canadian Council on Animal Care regulations for the use of experimental animals and were reviewed and approved by the institutional animal ethics committee.

Preparation of the cannulae. Each cannula can be prepared in less than 5 min. All components of the cannula were steam-autoclaved individually then glued together under sterile conditions immediately prior to surgery by using a high-viscosity instant gel adhesive (409 SuperBonder, Loctite Corp., CT).

The 22G cannulae were made of Vialon biomaterial, a proprietary polyurethane used to make several currently marketed vascular catheters (Intracath, Becton Dickinson, Sandy, UT). The i.v. catheter placement unit was dismantled, and the Vialon biomaterial tubing was trimmed to a length of 10 cm. The last 6 mm of the tubing was held over the tip of a hot soldering iron to curve it to an angle of 45° (Figure 1). Keeping the tip of the modified cannula towards the left, the dorsal surface of the cannula was ink-marked over its entire length to indicate the proper orientation of the cannula during its insertion. Two small holes (<0.5 mm in diameter) were then made within 2 mm of the tip of the cannula (Figure 1) by using a hot 30G wire wrapped around the tip of the soldering iron and the aid of a dissecting microscope. These holes ensure that blood can be withdrawn despite accidental obstruction of the tip of the cannula (e.g., blood clot, impinging against the artery wall, etc.). The cannula was also examined to ensure that the holes did not present any rough edges, which could cause small lesions to the carotid ar-

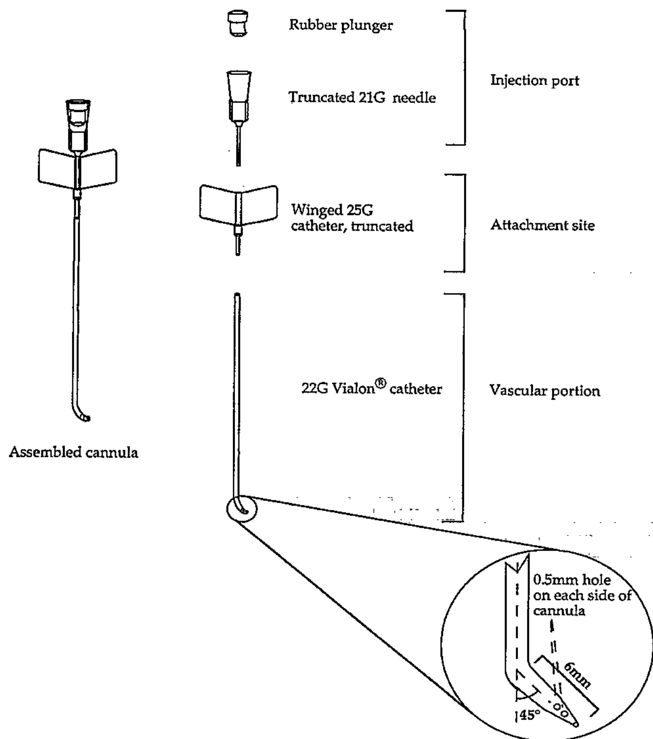

FIG. 1. Design of chronic arterial cannula. Inset shows details of the tip of the cannula. For the sake of clarity, the half-moon of self-adherent Velcro® pile was removed from the winged catheter.

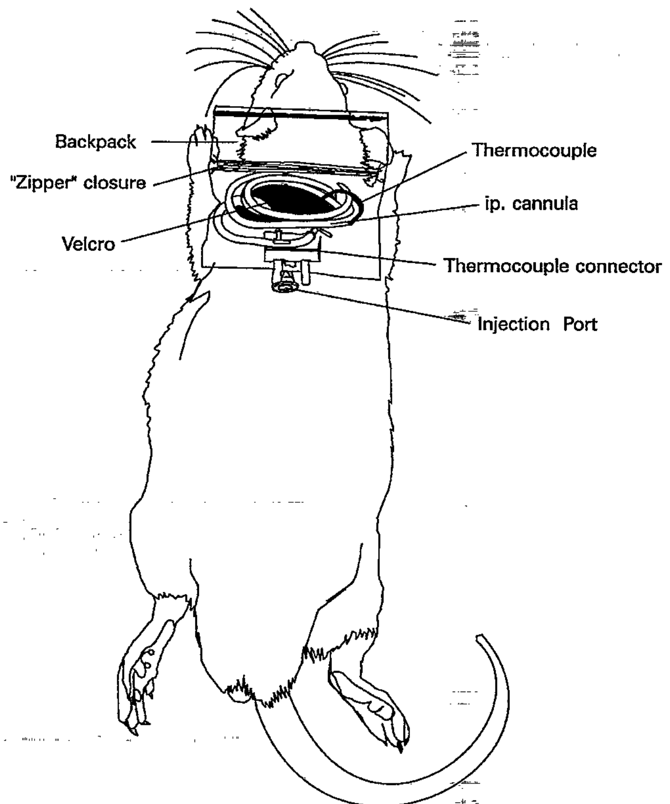

FIG. 2. Location of backpack on the experimental animal.

tery during its insertion. Any rough edges were smoothed by using the hot 30G wire. This technique required some practice to optimize the proper contact time with the Vialon tubing and avoid undesirable deformation or melting of the tubing.

The beveled portion of the 25G needle of a winged catheter (E-Z set, Becton-Dickinson, Sandy, UT) was milled to 5 mm by using a Dremel moto-tool (model 395, Racine, WI). The macrobore tubing extension of the winged catheter was then reduced to 2 mm in length, and the Vialon cannula was glued over the trimmed portion of the 25G needle. An injection port was made by inserting the rubber plunger of a 1 cc syringe (Tuberculin, Becton-Dickinson, Sandy, UT) into the hub of a 21G needle milled to 1 cm. The injection port was then glued into the macrobore tubing extension of the winged catheter to fit snugly over its 25G needle (Figure 1).

Two 2.5 cm disks of self-adherent Velcro® (hooks and pile disks) were cut in half. The half-moon of Velcro® pile was then applied firmly to cover the winged catheter, with the cut edge oriented towards the injection port; this material was used to secure the optional backpack. Subsequent autoclaving ensured vulcanization of the Velcro® pile onto the winged catheter. The assembled cannula weighs approximately 300 mg, has a dead space of less than 60 uL, and costs about US $2.50/unit.

Preparation of the optional backpack. Low-density polyethylene bags (4.0 cm x 5.0 cm; Sigma Chemical Company, St. Louis, MI) with a double-track 'zipper' closure were modified to make a backpack (Figure 2). A half-moon of Velcro® (with hooks) was firmly applied on one side of the bag. The bag was then positioned on top of the sutured winged catheter in such a way as to allow free access to the injection port lying directly under the polyethylene bag. The backpack weighed approximately 750 mg. In our laboratory, a 40G copper-constantan thermocouple (Type T, Omega Engineering Inc., Stamford, CT) as well as an in-house produced intraperitoneal cannula (Silastic tubing, 0.76 mm ID, 1.65 mm OD; Dow Corning Medical, Mississauga, ON) were stored in the backpack for as many as 3 days prior to experimental induction of peritonitis. The i.p. cannula and thermocouple were implanted after the arterial cannulation was completed.

Surgical procedures. All surgical procedures were performed under aseptic conditions, with the animals under general anesthesia (2.0% halothane, 1:1 oxygen:nitrous oxide). The craniodorsal and ventral cervical regions were shaved then cleansed with povidone-iodine and alcohol. A 1 cm longitudinal skin incision was made on the dorsum of the neck, just caudal to the ears; a similar one was made over the left carotid artery. The cannula was tunneled subcutaneously around the neck from the dorsal skin incision to the ventral cervical incision; it was then filled with heparinized saline and introduced into the left carotid artery by using standard surgical procedures (22). The marking of the dorsal side of the cannula and its curved tip ensured insertion into the thoracic aorta and not into the left ventricle. Mean arterial pressure (Pressure monitor model SP1405, Gould Statham, Oxnard, CA) was monitored as the cannula was advanced into the blood vessel to confirm the proper positioning of the cannula. The cannula was ligated in the left carotid artery, just below the carotid artery bifurcation, by using nonabsorbable 4-0 silk sutures (Johnson & Johnson, Mississauga, ON). For each animal, all surgical procedures were completed in 10 min.

In our studies on the inflammatory response to intra-abdominal infection, the abdominal region was also shaved and cleansed with povidone-iodine and alcohol. The i.p. cannula was then inserted 3-cm deep into the abdominal cavity through a 2-mm incision, located 5 mm below the xiphisternal cartilage. The distal end of the i.p. cannula was then tunneled subcutaneously along the spine to emerge through the skin incision on the dorsum of the neck. The i.p. cannula was inserted into the polyethylene bag through a 3-mm puncture located ventrally below the 'zipper' closure of the bag. The subminiature male connector of the thermocouple was inserted in the polyethyl-

ene bag and its prongs were pulled through the bottom of the bag (Figure 2). The thermocouple was also carefully pulled out of the bag through the 3-mm puncture. The thermocouple was inserted into the skin incision on the dorsum of the neck then pulled subcutaneously along the same path as that for the i.p. cannula; a 3-cm length of the thermocouple was inserted into the abdominal cavity. The i.p. cannula and thermocouple were anchored in place by suturing them to the muscles of the neck and abdomen with nonabsorbable 4-0 nylon sutures (Johnson & Johnson, Mississauga, ON). On the day of the induction of peritonitis, the i.p. cannula was pulled out of the backpack through a 2-mm puncture located dorsally below the 'zipper' closure of the polyethylene bag. To protect it from the rats for the 12-h experiment, the i.p. cannula was then inserted into a stainless steel tether.

All skin wounds were closed by using nonabsorbable 3-0 silk sutures (Johnson & Johnson, Mississauga, ON), and topical antibiotic (Hibitane, Ayerst Lab., Montréal, QC) was applied. The winged cannula was then sutured by using 4-0 nylon (Johnson & Johnson, Mississauga, ON) in four locations to the skin above the shoulders of the rats so that it laid out of their reach; care was taken not to puncture the lower surface of the skin to prevent infection and irritation. The animals received a s.c. injection of sterile saline (30 mL/kg body weight) and were given a single dose of analgesics (buprenorphine i.m., 0.05 μg/g body weight). Arterial cannulae were flushed daily with sterile heparinized saline (30 U/mL).

Experimental protocol. Twelve rats were cannulated (CAN), and six underwent sham surgical procedures (SHAM). A group of six healthy, unmanipulated animals (HEA) was also included in the study to provide baseline values for the different parameters measured. Arterial blood samples (300 uL) were obtained at day 0, 4, 7, and 10 after cannulation from all CAN animals. Blood samples (300 uL) were obtained by transection of the tail from SHAM rats at the same time points. Blood samples were obtained at the same time (8 a.m. to 9 a.m.) throughout the study period. To assess bacterial contamination, a small volume of blood was processed by using standard microbiological procedures. For six of the CAN rats, total leukocyte and erythrocyte cell counts were determined by using automated methods (Coulter JT, Coulter Corp., Hialeah, FL). TNF-α and IL-6 levels were determined in the samples taken from another group of six CAN animals by using commercially available ELISA kits specific for rat cytokines (Medicorp, Montréal, QC). A gross necropsy examination was performed 10 days after surgery in all cannulated animals.

Statistics. Data were analyzed by using the F test. When the F value was significant ($p < 0.05$), Cochran t-tests were used to compare the individual time points to baseline values. Student's *t*-tests were used to compare CAN and SHAM animals. All data are expressed as mean ± SEM.

Results

The animals did not appear stressed when the experimenter was handling the injection port to withdraw blood. Though weight gain was reduced ($p < 0.05$) for 4 days in both CAN and SHAM compared to HEA, normal growth had resumed by day 10 in both groups (Figure 3). There was no systemic bacterial contamination or infection of the wounds in any of the cannulated animals. There was also no deleterious effect on hind limb function resulting from the slight reduction (< 20%) in cross-sectional area of the lumen of the thoracic aorta. Careful examination at necropsy revealed no gross lesions of the aortic vessel or other organs. However, there was deposition of some fibrin around the left carotid artery or along the aortic arch in 2 of the 12 experimental animals.

The arterial cannulae remained patent for the study period in all animals. Figures 4 and 5 depict the changes in hematological and immune parameters for 10 days in the various groups of animals. Compared to SHAM animals, CAN animals had a small increase in leukocyte counts after 10 days (Figure 4A), but this trend did not achieve statistical significance ($p = 0.07$). There were no significant differences in red blood cell counts between the groups throughout the study period (Figure 4B). Neither the sham operation nor cannulation affected TNF-α levels (Figure 5A). By day 7, IL-6 levels in both CAN and SHAM rats showed a small but significant decrease ($p < 0.05$) compared to those in HEA animals; however, these levels had returned to normal at day 10 in both groups (Figure 5B).

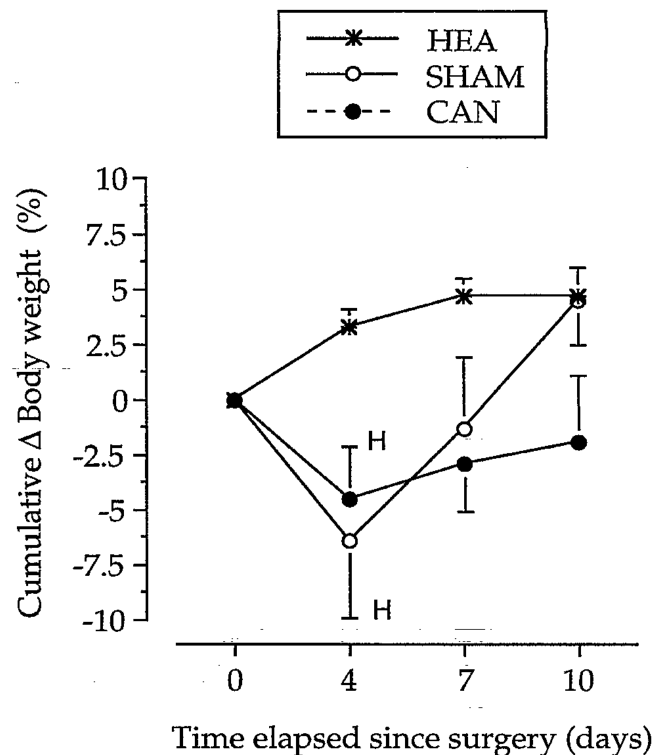

FIG. 3. Cumulative changes in body weight in healthy, unmanipulated animals (HEA; n=6), sham-operated (SHAM; n=6), and cannulated animals (CAN; n=6) during the study period. [H] significantly different from HEA. Data are presented as mean ± SEM.

Discussion

The technique described in this report was simple, practical, and reliable in facilitating the withdrawal of blood for at least 10 days in conscious, unrestrained rats. The results presented also show that our method has no or minimal effect on some of the hematological and immune parameters commonly measured to assess the immune status of experimental animals (22–24). Furthermore, the optional backpack allowed storage of other experimental devices for intraperitoneal infusions and measurement of body temperature. We have used this backpack technique for as many as 3 days prior to infusing rats intraperitoneally with a bacterial inoculum; the animals were apparently unaware of carrying the backpack (unpublished data). Our technique may also be useful for storing other types of experimental devices designed for acute, intermittent, or short-term infusions and injections. Therefore, our new cannula design may be a valuable addition to the various techniques of catheterization currently used.

The arterial cannulae were flushed daily in the present study; they also remained patent when maintenance flushing was performed only twice weekly (unpublished data). The favorable

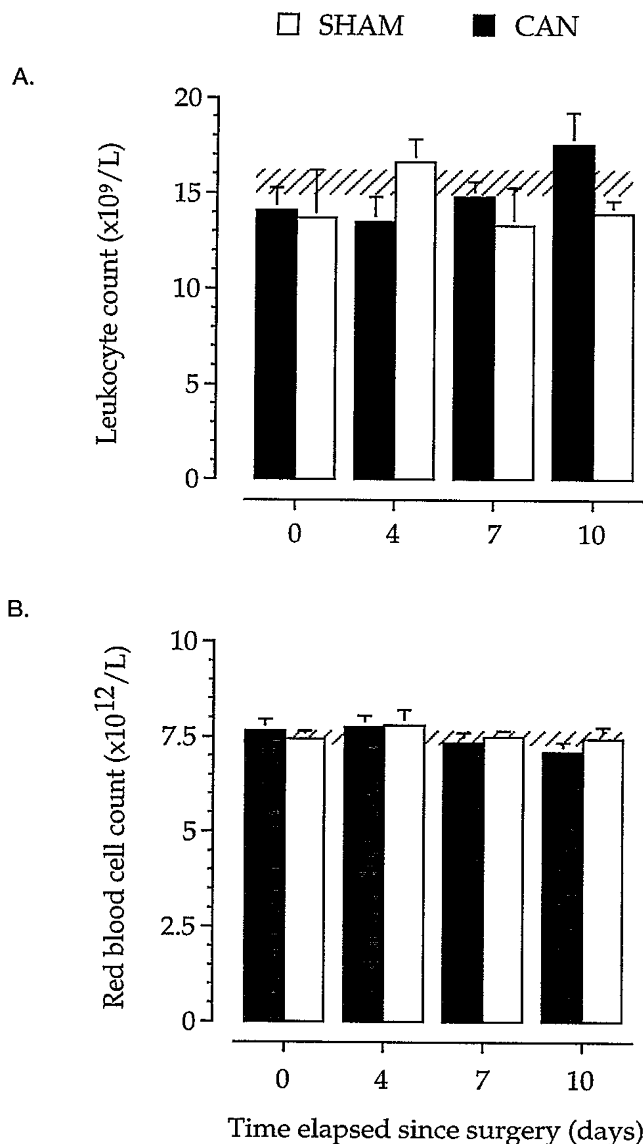

FIG. 4. Changes in white blood cell count in sham-operated (SHAM; n=6) and cannulated animals (CAN; n=6) during the study period. Hatched area represents baseline values obtained from six healthy, unmanipulated animals (HEA). Data are presented as mean ± SEM.

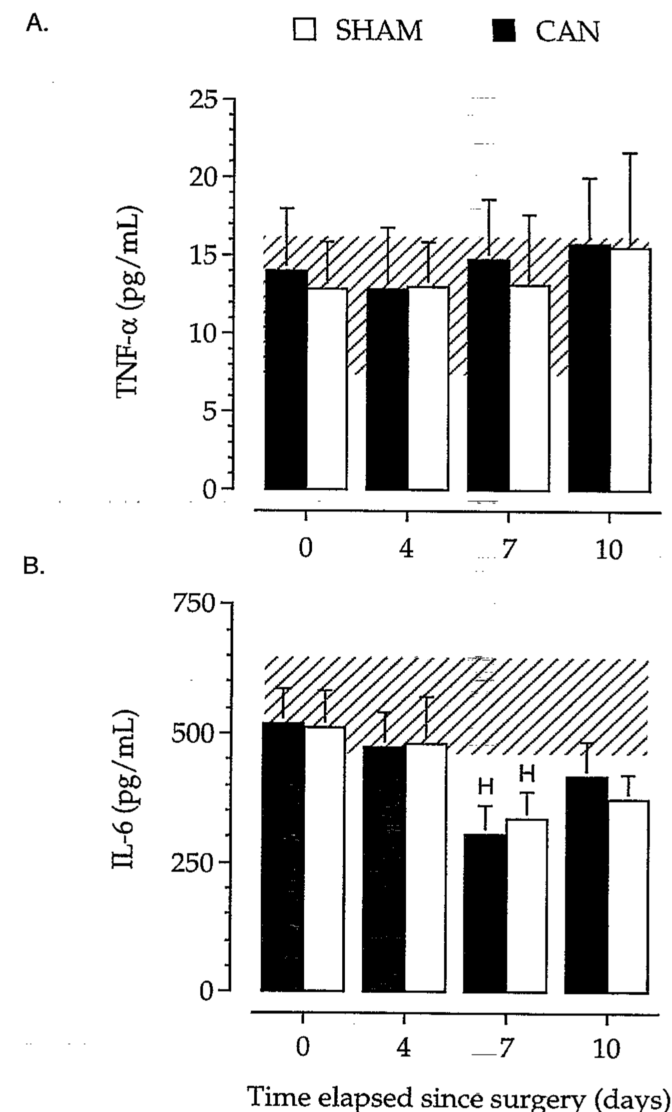

FIG. 5. Changes in cytokine levels in sham-operated (SHAM; n=6) and cannulated animals (CAN; n=6) during the study period. Hatched area represents baseline values obtained from six healthy, unmanipulated animals (HEA). [H] significantly different from HEA. Data are presented as mean ± SEM.

patency of our arterial cannulae can be partly attributed to the type of tubing used: Vialon biomaterial is resistant to blood clotting as well as essentially non-reactive to body tissues, unlike the polyethylene (1–3, 18, 19) or polyvinylchloride (8) tubings that are commonly used to design rodent cannulae. Torres-Molina et al. (6) have also reported a long patency period for their venous cannula when constructed of Vialon biomaterial. Silicone rubber tubing shares the same characteristics as Vialon and has been used successfully in designing various types of cannulae for serial venous blood sampling in rats (9, 12–17). Cannulation of the thoracic aorta via the left carotid artery using silicone tubing is considered impractical due to anatomical problems and the extreme flexibility of this material. However, Tsui et al. (23) have suggested inserting a monofilament line into the silicone rubber tubing to provide internal support for a cannula, thus facilitating its insertion into the thoracic aorta. Whether or not the patency period that we observed would be preserved if such a modified silicone rubber tubing were substituted for the Vialon tubing of our cannula remains to be assessed.

Patency of our cannulae was also enhanced by the presence of holes near the tip of the cannula. In preliminary trials, single-holed polyurethane cannulae remained patent in approximately 75% of the animals for at least 10 days, whereas polyethylene or polyvinylchloride cannulae did not remain patent for longer than 2 or 3 days despite their proper location in the thoracic aorta. Polyethylene and polyvinylchloride tubings also tended to deform when the hot wire technique was used to perforate them. Because using a needle to puncture these tubings was not effective, we do not recommend the use of these materials for our application.

TNF-α and IL-6 are two important cytokines with a wide array of immunoregulatory, metabolic and pro-inflammatory activities. Thus, elevated levels of these cytokines have been observed following various injuries, infectious diseases, and surgical procedures (24, 25). Our finding of low and comparable levels of TNF-α and IL-6 in the cannulated and healthy animals throughout most of the 10-day study suggests that there was no systemic inflammatory response after the arterial cannulation. In contrast, there was a local inflammatory response, as suggested by the deposition of fibrin around the left carotid artery. Similar local inflammatory response has also been reported 7 days after subcutaneous implantation of polyurethane cannulae in a rab-

bit model (26). We have not attempted to keep the cannulae functional for longer than 10 days; however, we suspect that this local inflammatory response may have eventually worsened to the point of preventing withdrawal of blood, as reported for other types of chronic cannulae (17, 18). It is also noteworthy that while there was no significant effect of the cannulation procedures on the immune status of the animal, ligation of the left carotid artery just below the carotid bifurcation as performed in the present and other studies (3, 5, 26) may interfere with the baroreceptor and chemoreceptor functions of the animal on the left side of the body. Therefore, a different blood vessel should be cannulated by investigators interested in studying and/or preserving these functions at or near normal levels.

Typically, the free end of venous or arterial cannulae emerges on the dorsal cervical area; the emerging cannula is then kept short (5, 17, 18), or an extension is attached to it to allow blood withdrawal (9, 16, 26, 27). Several investigators have also reported methods for attaching cannulae onto the skull of rats (12, 13, 27). To our knowledge, there was no report of a technique for fixing a cannula and/or other experimental devices onto the shoulders of the experimental animal. Forsling and Wells (28) have designed a rodent jacket that held their venous and arterial cannulae in special pockets. However, we were reluctant to use any harness, not only because it induces stress by restraining movement (29) but also because it interfered with the patency of our i.p. cannula (personal observation). The method described here has proven reliable and satisfactory: the injection port of the cannula lies protected between the scapulae when not in use but affords easy access during blood sampling. Some investigators are reluctant to suture or otherwise fix any supporting device to the dorsum or head of experimental animals, but our rats did not seem aware of carrying the lightweight backpack, which was intact after the study period. Furthermore, the animals did not show any sign of stress when blood samples were taken or when the thermocouple connector was handled to measure i.p. temperature. In fact, satisfactory quietness was confirmed in preliminary experiments by a stable pulse rate and blood pressure. We believe that this simple, inexpensive backpack also allows a great freedom of movement to the animal.

In summary, this simple, easily built cannula allows blood sampling at least 10 days while providing a novel attachment site for a lightweight, unobstructive backpack. Such an assembly may be useful in those experimental designs precluding the use of conventional postoperative restraint methods and requiring more than one experimental device to be implanted and protected from the animal. Use of this method is probably not limited to the circulation. With just minor modifications to the tip of the cannula and/or its length or size, a cannula of our design could be used to facilitate intermittent injection of drugs or other substances into hollow viscera or body cavities (e.g., the stomach or peritoneal cavity) in larger rodents or even rabbits.

Acknowledgments

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