A novel alternative placement site and technique for totally implantable vascular access ports in non-human primates

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Introduction

Intravenous (IV) catheterization for drug administration and blood sampling is among the most common procedures large animals undergo in the experimental laboratory animal setting. When repeated entry into the venous circulation is required over a long period, vascular access ports (VAPs) are widely used to preserve vascular access, improve safety, and increase animal comfort [1–11]. VAPs generally enhance the well-being of non-human primates (NHPs), allowing them to participate without restriction in enrichment programs and social group activities without the restrictions inherent in conventional jacket and tether systems. The use of VAPs lowers stress for the animal, not only adding to the animal’s well-being but also improving the outcome of experiments and the quality of data gathered. Moreover, VAPs do not restrict any species’ typical behaviors, such as social grooming, playing, climbing, and swimming [20]. VAPs provide a simple and painless means of drawing blood or delivering drugs and nutrients, thereby encouraging cooperation with animal handlers. In our experience, NHPs become fully accustomed to routine handling of their VAP after appropriate training.

The two traditional methods of VAP implantation in NHPs are jugular venous cutdown (JVC) and tunnel, and femoral venous cutdown (FVC) and tunnel [1, 12, 13]. Previously, we employed the FVC approach, making a longitudinal incision below the inguinal liga-

Keywords
blood collection – central venous catheterization – indwelling catheter – macaca – refinement

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Accepted December 27, 2008.

Abstract

Background Two novel approaches to implanting a central venous catheter port in non-human primates (NHPs) using peripheral insertion are presented and compared.

Methods Sixty vascular access ports (VAPs) implants were attempted in 52 NHPs by saphenous vein puncture (n = 20) or saphenous vein cutdown (n = 40).

Results Fifty eight procedures were successful. Eighteen of 20 VAPs were successfully placed using saphenous vein puncture, and 40 of 40 using saphenous vein cutdown. There were no significant differences between procedures. Mean implantation times were similar between groups. At explant or study endpoint, all 58 VAPs were patent.

Conclusions Vascular access port implantation by saphenous vein puncture or saphenous vein cutdown is safe and effective in NHPs. It is less invasive than conventional procedures, has fewer complications, provides outstanding patency, and reduces surgery time. Furthermore, it allows for cooperative in-homecare VAP use, minimizing handling stress. We recommend these refined methods for long-term vascular access in NHPs.
ment to access the femoral vein and tunneling the catheter to a second incision housing the port on the posterior chest wall [14]. The relative invasiveness of two separate incisions with a lengthy tunnel requires general anesthesia. Also, complications are common and include infections, mechanical occlusion, thrombotic occlusion, dehiscence or erosion, and even death [1, 4, 7, 10, 12, 14–17]. Postural movement of the NHP can restrict the catheter (at the hip joint in FVC or bending acutely at the port head in JVC), resulting in mechanical failure [1]. Ordinary tension placed on an inguinal incision during normal movement is associated with post-operative irritation and discomfort [14]. The inguinal region is expected to have higher bacterial densities and potential for infection than other sites [18, 19]. Complications often require surgical revision or removal, which is costly and presents an additional burden and health risk to the animal.

Mindful of these limitations, we searched for alternatives to conventional VAP implantations that would minimize surgical invasiveness, permit appropriate socialization by not restricting the NHP in any way, and support in-homecare training regimens that minimize stress. Here we describe a new technique to achieve long-term central venous access, single incision peripheral insertion (SIPI), and evaluate two alternative approaches to it: saphenous vein puncture and saphenous vein cutdown. We recommend the application of this refined technique when long-term venous access is needed in the experimental protocol.

Materials and methods

This study was approved by the University of Minnesota Institutional Animal Care and Use Committee and conducted in compliance with the Animal Welfare Act adhering to principles stated in the Guide for Care and Use of Laboratory Animals.

Animals

Between September 2006 and February 2008, 52 (35 male/17 female) apparently healthy NHPs underwent VAP implantation. We used NHP species that are commonly used in biomedical research; 34 cynomolgus macaques (Macaca fascicularis), 13 rhesus macaques (Macaca mulatta), and 5 baboons (Papio anubis). The macaques weighed 2.89–5.62 kg and the baboons weighed 12.3–16.7 kg. Their age ranged between 40 and 75 months at time of VAP placement. All animals were purchased from institutionally approved commercial vendors. They were housed in pairs or in small groups of the same gender. They had free access to water and were fed biscuits (High-Protein Monkey Chow 5045; Purina Mills Inc., St. Louis, MO, USA) based on weight. Their diet was liberally enriched with fresh fruits, vegetables, grains, beans, nuts, and nutritional supplements. The animals participated in an environmental enrichment program that included social play, toys, music, movies, and regularly scheduled access to large-exercise and swimming areas. To facilitate cooperation in blood collection and drug administration, all animals were trained to present the port in-homecare, and all became accustomed to routine handling, avoiding the need for restraint or sedation during routine procedures.

VAPs

Port type and the catheter size are described in Table 1 and illustrated in Fig. 1. VAPs were sufficiently large to enable palpation while still respecting the amount of subcutaneous space necessary to accommodate the port head reservoir and the placement site. We selected hydromer-coated (Hydrocoat) polyurethane catheters for their resistance to kinking and their relative strength. All VAPs were visually inspected and pressure-tested with saline for defects before implantation.

Surgical technique

Food was withheld for 16 hours before surgery. Surgeons and assistants prepared by scrubbing with Avagard chlorhexidine gluconate 1% solution and...
ethyl alcohol 61% (3M, St. Paul, MN, USA) and wore sterile impervious gowns, latex surgical gloves, masks, and caps. Prophylactic antibiotic, cefazolin 50 mg/kg, was administered intramuscularly (IM) before surgery. Animals were sedated using Telazol® (Wyeth, Fort Dodge, IA, USA), 6 mg/kg IM, followed by a local anesthetic block. The surgical area was clipped and widely prepped with Technicare (Care-Tech Laboratories Inc., St. Louis, MO, USA) and then draped for aseptic surgery.

Procedure for saphenous vein puncture (group A, n = 20 attempts)

For the percutaneous saphenous vein puncture approach, the NHP was placed in a prone position. The skin overlying both the anticipated venipuncture site and the port pocket was infiltrated with 1% lidocaine, creating a weal underneath the skin. The saphenous vein was visualized and occluded (Fig. 3A). A 16-gauge peripheral IV catheter (Jelco®, Smiths Medical, Kent, UK) was used to penetrate the vein and served as the introducer. Once flow of blood into the introducer hub confirmed vein penetration, the introducer was advanced into the vein and the needle withdrawn. The introducer was flushed with normal saline from a 3 ml syringe (Fig. 3B). A flexible J guidewire was advanced through the 60 cm polyurethane central catheter, just 2–3 mm after the tip. Prior to insertion, the catheter was measured outside the body and marked to reach a length 6–8 cm proximal to the iliocaval bifurcation. Measurement was best accomplished by palpating the iliac crest and lowest rib; proper catheter length was approximately midway in between at the level of the umbilicus (Fig. 2). The catheter over guidewire was advanced through the introducer and then advanced centrally to the inferior vena cava (Fig. 3C). Special attention was given to avoid handling the catheter with sharp-toothed instruments. Resistance was overcome by rinsing the catheter with normal saline and in some cases adjusting the position of the hip from extended to flexed while gently advancing the catheter. When the catheter reached the mark, the guidewire was removed and the catheter was tested, then flushed with normal saline. The introducer was removed by sliding back over the catheter. Firm pressure was applied at the entry site for 90 s. A 1–1.5 cm port pocket incision was made approximately 6–10 cm proximal to the insertion site on the lateral thigh. Blunt dissection was used to create a
conventional port pocket to accommodate the port head. A long-curved forceps was used to create a tunnel just wide enough to accommodate the tips from the incision to the catheter (Fig. 3D). Gentle blind blunt dissection was performed to free the vessel from the overlying skin so the catheter could be grasped and pulled through the tunnel (taking care to pull in from the skin puncture versus out of the vessel). It was not necessary to anchor the catheter at the vessel with a ligature. The catheter was trimmed and attached to the port head using non-absorbable suture and the sheath was slid up to the port head, providing added security (Fig. 3E).

The port was placed into the pocket. Polyglactin braided suture, a synthetic absorbable surgical suture, was selected for closure. The incision was closed in two layers, the superficial fascia with continuous sutures followed by continuous subcuticular sutures (Fig. 3F). A topical skin adhesive (Dermabond, Ethicon Inc., NJ, USA) was applied as a protective barrier over the incision.

Procedure for saphenous vein cutdown (Group B, n = 40 attempts)

For the saphenous vein cutdown approach, the NHP was placed in a prone position. The skin overlying both the anticipated incision site and the port pocket was infiltrated with 1% lidocaine, creating a weal underneath the skin. A vertical 1–1.5 cm incision was made lateral to the saphenous vein distal to the knee (Fig. 4A). Using blunt dissection, the saphenous vein was visualized and looped with two separate absorbable sutures, one proximal and one distal (Fig. 4B). A flexible J guidewire was advanced through the 60 cm polyurethane central catheter, just 2–3 mm after the tip. Prior to insertion, the catheter was measured outside the body to reach 6–8 cm proximal to the iliocaval bifurcation and marked. Measurement was best accomplished by palpating the iliac crest and lowest rib and using the midpoint between them, at the level of the umbilicus, as an approximation to the proper catheter length (Fig. 2). The saphenous vein was then partially transected with a No. 11 blade in a transverse fashion (Fig. 4C). Back-bleeding from the proximal end of the saphenous vein was controlled by applying traction to the proximal suture and the distal suture was then tied off. The catheter over guidewire was then passed proximally into the lumen of the partially transected saphenous vein and advanced centrally into the inferior vena cava (Fig. 4D). Special attention was given to avoid handling the catheter with sharp-toothed instruments. Resistance to advancing the catheter was overcome by rinsing the catheter with normal saline and in some cases adjusting the position of the hip from extended to flexed while gently advancing the catheter. When the catheter reached the mark, the guidewire was removed and the catheter was tested and then flushed with normal saline. The proximal suture looping the saphenous vein was removed and
the distal tie trimmed. Using the same incision as a starting point, blunt dissection was used to create a conventional port pocket to accommodate the port head (Fig. 4E), 4–10 cm proximal on the lateral thigh. The catheter was trimmed and attached to the port head using non-absorbable suture, and the sheath was slid up to the port head, providing added security. Polyglactin-braided suture, a synthetic absorbable surgical suture, or polydioxanone monofilament, a synthetic absorbable surgical suture, was selected for closure. The port was placed into the pocket and the incision closed in two layers, the superficial fascia with continuous sutures followed by continuous subcuticular sutures (Fig. 4F). A topical skin adhesive (Derma-bond, Ethicon) was applied as a protective barrier over the incision. Finally, the VAP was flushed with normal saline and locked using a catheter lock solution that contained an anticoagulant plus a thrombolytic [200 U heparin sodium, diluted with 0.5 ml normal saline with 2000 IU Urokinase (ImaRx Therapeutics, Inc. Tucson, AZ, USA)].

Procedure for vascular access port removal (n = 6)
The NHP was placed in a prone position as for VAP placement. The skin overlying and around the port was infiltrated with 1% lidocaine. A 1–1.5 cm incision was made just distal to the port. Blunt dissection was used to free any adhesions to the port and expose the proximal end of the catheter. The port and catheter were then easily removed by gentle traction. Pressure was applied to the venotomy for several minutes. Skin was minimally approximated to promote rapid healing while still allowing for potential drainage.

Results
Fifty-two apparently healthy NHPs underwent an attempt to implant a port system using a single incision. In 17 (Group A), saphenous vein puncture was used, and in 35 (Group B), saphenous vein cutdown was used. In two of 17 animals in Group A, the catheter failed to advance in a first attempt, but a subsequent attempt on the opposite side was successful. In six animals (one in Group A and five in Group B, as detailed below), the initial VAP was removed and subsequently replaced with a second VAP. This resulted in 60 total attempted placements. Overall success rate for placement in Group A was 90% (18/20). All 40 attempts in Group B were successful, without intra-operative complications (100% overall success rate). The difference in success rate between the two groups was not statistically significant (Chi-squared test).
In Group A, average operative time to insert the VAP system was 43 ± 13 minutes (mean ± SD; range: 26 to 73 minutes). In Group B, it was 39 ± 8 minutes (range: 27 to 68). All animals recovered quickly, uneventfully, and without complications from anesthesia. Primary complications are described in Table 2. They led to secondary complications in six animals (one in Group A and five in Group B) which are given below.

In Group A, two cases (11.1%) developed a stitch abscess. If suture was apparent it was removed. The abscess resolved in one case, while in the other case it persisted and subsequently dehisced, requiring surgical revision.

In Group B, five cases (12.5%) developed a stitch abscess. If suture was apparent it was removed. The abscess resolved spontaneously in two cases without additional surgical intervention. The other three cases developed a bacterial infection, two with co-occurring dehiscence resulting from infection that was culture positive. In all three this necessitated removal of the device. In two cases (5%) a hematoma developed; both animals developed a bacterial infection requiring removal of the device.

One case in Group B manifested a 180° ‘flipping’ of the port head reservoir within its subcutaneous pocket. Surgical revision was not necessary as it could be palpated into a correct position. In one case dehiscence was observed, and attributed to the animal removing the surgical glue and the subcuticular sutures. Surgical staples were used to successfully repair and prevent additional disruption to the wound site. This case resolved without further complication.

In one case in Group B the sheath eroded through the skin and the device was removed.

In all six cases requiring device removal, devices were easily and successfully removed under sedation with local anesthetic block.

There were no infections in Group A and five in Group B, for an overall infection rate of 8.6%. Site samples of infected animals revealed coagulase-positive staphylococcus (5 of 5) and non-fermentative gram-negative bacilli (3 of 5). There was no indication of systemic progression in any of the animals. In Group B animals, the closing suture directly impacted the development of primary complications and subsequent infection. Eight animals were closed using polyglactin-braided suture and 32 animals using polydioxanone monofilament. The association between subsequent infection and suture type was analyzed in a bivariate fashion. The infection rate in Group B animals was 3.1% when closure was performed with polydioxanone monofilament, and 50% in animals closed with polyglactin braided. This difference between the two types of sutures was statistically significant \((P = 0.0035, \chi^2\text{-test})\). This phenomenon is ascribed to the fact that monofilament sutures resist harboring organisms that may cause suture line infection \([21]\).

All animals were monitored for compliance (defined as not disturbing the wound site) during the follow-up period and evaluated for relative comfort at the single incision site. No irritations or evident disruption to the wound site were observed in 89.7% of animals.

During follow-up, VAPs were routinely accessed (mean ± SD, 8.8 ± 14 days, range: 1–111 days) for blood sampling, fluid administration, and drug administration. For animals that were sacrificed (n = 37) as part of the protocol for primary study (see limitations), catheters were assessed for patency, and at necropsy, catheter position and condition were examined. Post-mortem observation of catheters after dissection consistently revealed an approximate 3–5 cm change in tip location due to movement of the leg from an extended to flexed position. The catheter and vessel were assessed for catheter-induced fibrin formation. One case (2.7%) manifested moderate fibrin cuff formation but it did not impede the catheter. The patency rate for all animals (n = 58) was 100% through the follow-up period (median: 97 days, range: 36–217 days).

**Study limitations**

After port placement NHPs were subsequently enrolled in transplantation experiments. The access schedule, follow-up period, and endpoint varied among the animals. Because of this limitation we were unable to assess maximum catheter longevity or durability.

### Table 2 Incidence of primary complications in NHPs after single-incision peripheral insertion placement of vascular access ports

<table>
<thead>
<tr>
<th>Primary complication</th>
<th>Group A (n = 18)</th>
<th>Group B (n = 40)</th>
<th>Total (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stitch abscess, n (%)</td>
<td>2 (11.1)</td>
<td>5 (12.5)</td>
<td>7 (12.1)</td>
</tr>
<tr>
<td>Hematoma, n (%)</td>
<td>0 (0)</td>
<td>2 (5.0)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>Migration/malposition of port, n (%)</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Dehiscence, n (%)</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Erosion, n (%)</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Catheter occlusion, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

NHP, non-human primates; VAP, vascular access ports.

Complications were assessed in VAPs successfully placed using the saphenous puncture technique (group A) and VAPs successfully placed using the saphenous cutdown technique (group B).
because of complications, it is easily carried out under sedation without major dissection. Second, the lack of fixation to the vessel allows the catheter to move freely within the inferior vena cava (IVC). The large ratio between the small catheter (3.5F) and the relatively large diameter of the IVC suggests that the catheter tip is likely to lie parallel to and not impinge on the IVC vessel wall. Third, stasis can occur between the catheter and the vessel wall if the catheter tip is fixed [28]. The constant movement of the tip decreases the possibility of prolonged tip contact with the vessel wall and prevents disturbance of normal blood flow. Remarkably, we observed fibrin deposition at necropsy in only one case. This led to the conclusion that common thrombotic complications were precluded, including intraluminal thrombus, mural thrombus, fibrin sheath thrombosis, and fibrin tail thrombosis, which is often the main reason for occlusion [29].

When the port head is placed on the chest wall as in JVC/FVC, the NHP typically requires manual or chemical restraint for access. Restraint is a stressful event associated with changes in physiologic and metabolic parameters [30–38]. For instance, it has been shown that adult rhesus macaques develop a quick and significant increase in serum cortisol concentration as a response to venipuncture performed away from their familiar home cage, while this response is absent when they are venipunctured in their own cages [39, 40]. With the SIPI approach, we train animals to offer the leg with the port voluntarily in their familiar home cage for access and handling, eliminating the need for chemical or manual restraint.

In conclusion, SIPI has a low complication rate and is less invasive and requires a shorter period of surgery than traditional approaches of VAP implantation. It shows excellent patency. Most importantly, SIPI allows for cooperative in-homecage use of the VAP, minimizing handling stress. Based on our success with SIPI techniques, we recommend this method as a refined approach if long-term vascular access is needed.

### Discussion

We developed the SIPI approach, with either saphenous vein puncture or saphenous vein cutdown, to avoid many of the challenges of JVC and FVC (Table 3). Infectious complications associated with JVC/FVC ranges between 13% and 30% [12, 14, 22, 23] and those for jacket and tether systems between 25% and 30% [24, 25]. The overall rate of infection for SIPI found in the present study, 8.6%, is substantially lower. There were no infections in Group A (saphenous vein puncture) and consideration of only monofilament suture cases reduced the infection rate in Group B (saphenous vein cutdown) to 3.1%, similar to infection rates reported in humans [26, 27]. The technique we describe was designed to be simple and to be easily performed under sedation with a local lidocaine block. Thanks to these simplifications, there is no need for general anesthesia and the average time of surgery is reduced by about 50% [14].

The catheter is never fixed to the vessel. This is valuable for three reasons. First, if removal is indicated because of complications, it is easily carried out under sedation without major dissection. Second, the lack of fixation to the vessel allows the catheter to move freely within the inferior vena cava (IVC). The large ratio between the small catheter (3.5F) and the relatively large diameter of the IVC suggests that the catheter tip is likely to lie parallel to and not impinge on the IVC vessel wall. Third, stasis can occur between the catheter and the vessel wall if the catheter tip is fixed [28]. The constant movement of the tip decreases the possibility of prolonged tip contact with the vessel wall and prevents disturbance of normal blood flow. Remarkably, we observed fibrin deposition at necropsy in only one case. This led to the conclusion that common thrombotic complications were precluded, including intraluminal thrombus, mural thrombus, fibrin sheath thrombosis, and fibrin tail thrombosis, which is often the main reason for occlusion [29].

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### Acknowledgments

This work was supported by grants from the National Institutes of Health (National Institutes for Diabetes, Digestive, and Kidney Diseases; National Institute of Allergy and Infectious Diseases) and the Juvenile Diabetes Research Foundation. We are indebted for the support provided by the Diabetes Research and Wellness Foundation, the Minnesota Medical Foundation, the Eunice L. Dwan Diabetes Research Endowment, the Children with Diabetes Foundation, and the Winston and Maxine Islet Transplant Fund.
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