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Original Research

Minimally Invasive Lumbar Port System for the Collection of Cerebrospinal Fluid from Rhesus Macaques (*Macaca mulatta*)

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Biomedical translational research frequently incorporates collection of CSF from NHP, because CSF drug levels are used as a surrogate for CNS tissue penetration in pharmacokinetic and dynamic studies. Surgical placement of a CNS ventricular catheter reservoir for CSF collection is an intensive model to create and maintain and thus may not be feasible or practical for short-term studies. Furthermore, previous NHP lumbar port models require laminectomy for catheter placement. The new model uses a minimally invasive technique for percutaneous placement of a lumbar catheter to create a closed, subcutaneous system for effective, repeated CSF sample collection. None of the rhesus macaques (*Macaca mulatta*; *n* = 10) implanted with our minimally invasive lumbar port (MILP) system experienced neurologic deficits, postoperative infection of the surgical site, or skin erosion around the port throughout the 21.7-mo study. Functional MILP systems were maintained in 70% of the macaques, with multiple, high-quality, 0.5- to 1.0-mL samples of CSF collected for an average of 3 mo by using aspiration or gravitational flow. Among these macaques, 57% had continuous functionality for a mean of 19.2 mo; 50% of the cohort required surgical repair for port repositioning and replacement during the study. The MILP was unsuccessful in 2 macaques, at an average of 9.5 d after surgery. Nonpatency in these animals was attributed to the position of the lumbar catheter. The MILP system is an appropriate replacement for temporary catheterization and previous models requiring laminectomy and is a short-term alternative for ventricular CSF collection systems in NHP.

Abbreviation: MILP, minimally invasive lumbar port

The collection of CSF from NHP is a frequent requirement of biomedical research, and single samples often are collected through lumbar or suboccipital puncture,[4,6,15] which is percutaneous, noninvasive, and generally effective. However, 52% of occipital samples and 24.6% of those from lumbar punctures in cynomolgus macaques (*M. fascicularis fascicularis*) had high RBC counts.[7]

When serial CSF sampling is a study requirement, lumbar catheterization is frequently used. The procedure is problematic in that CSF collection is unreliable. Even with successful catheter placement, externalization of the catheter with an awake but restrained NHP is not advantageous, catheter placement is temporary (thus necessitating repeated anesthesia for reinsertion), and reinsertion is not generally successful or advisable for 24 h after a previous catheterization.[1] Surgical placement of a CNS ventricular catheter system for CSF collection overcomes the limitations of lumbar catheterization for serial CSF collection but is a surgically intensive model to create as well as to maintain.[9,11-13] Previous lumbar port models have been developed but remain surgically invasive, requiring a laminectomy for catheter placement.[2,5] Our goal was to use a noninvasive, percutaneous lumbar-catheter placement procedure with the reliability and permanence of an indwelling port to develop our minimally invasive lumbar port (MILP) system. Developed for rhesus macaques, our current MILP system is an appropriate replacement for temporary lumbar catheterization and previous models requiring laminectomy and, in specific cases, is a suitable alternative for CNS ventricular catheters for serial CSF collection from NHP in short-term studies.

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Materials and Methods

The National Cancer Institute's IACUC approved this study. Adult male rhesus macaques (weight, 8.7 to 14.4 kg; *n* = 10) were socially housed (either paired with a single partner or placed into a group of up to 6 other adult rhesus) and cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*.[10]

The macaques were implanted with a polyurethane catheter (3-French, rounded tip, 60 cm, with removal beads; Solomon Scientific, San Antonio, TX) connected to a titanium low-volume port (SoloPort MIN LOVOL, Access Technologies, Skokie, IL). The beads were removed from the catheter prior to placement. The catheter and port system were sterilized by using ethylene oxide. For the size of macaques used in this study, we typically marked the catheter at 7.5 in. by using a sterile marker.

Surgical procedure. Fasted macaques were anesthetized with ketamine (10 mg/kg IM) and dexmedetomidine (50 µg/kg IM), intubated, and IV access was established (18-gauge, Insyte Autoguard, Becton Dickinson, Franklin Lakes, NJ). Anesthesia was maintained with isoflurane (1% to 2%) and oxygen at a total flow rate of 2 L/min. Temperature, heart rate, and oxygen saturation were monitored. Lactated Ringers solution was infused intravenously at a rate of 60 mL/h. The macaques were positioned in right lateral recumbency for aseptic surgery.

A skin incision was made over one of the vertebral spaces between L4 and L6. A Hustead epidural needle (18-gauge, 3.5 in.) was inserted into the vertebral space at a 90° angle until CSF flow was established. The catheter was inserted through the needle and advanced (Figure 1). When the catheter was not advanced readily in several macaques, the needle angle was adjusted to 110° and a small stylet was added, if needed. The catheter was advanced to the 7.5-in. mark, thus leaving 4 in. of the catheter within the spinal column once the epidural needle was removed. CSF flow through the catheter was confirmed, and the epidural needle was removed. A ligation clip was placed at the distal end of the catheter to prevent any additional loss of CSF during preparation for port placement (Figure 2). Prior to catheter attachment, the port was filled with normal saline (24-gauge Huber needle, Access Technologies). A subcutaneous pocket was made caudal to the catheter insertion site, the catheter trimmed to the attachment length, and the catheter attached to the port. CSF flow through the system was confirmed by accessing the port, the port was anchored to muscle (3-0 Prolene, Ethicon, Somerville, NJ), and 0.3 m bupivacaine (5 mg/mL, Hospira, Lake Forest, IL) was placed subcutaneously. The fascia and subcutaneous tissue were closed separately by using a combination of simple interrupted and simple continuous sutures (3-0 PDS, Ethicon). The skin was closed in a subcuticular pattern (4-0 PDS, Ethicon). CSF flow through the port was confirmed after the final skin closure (Figure 3).

Postsurgical treatment and evaluation. The macaques received cephalexin (25 mg/kg PO BID) for 7 d, buprenorphine (0.01 mg/kg IM BID) for 1 d, and ketoprofen (2.0 mg/kg IM QD) for 3 d. The macaques were anesthetized with ketamine (10 mg/kg IM) daily for 2 to 4 d to confirm CSF flow from the MILP system by using aspiration or gravitational flow. Once the incision was healed (usually within 1 wk after surgery), the macaque was returned to its social group or partner. Afterward, the functionality of the MILP was checked weekly for 1 mo and monthly thereafter for the duration of the study (21.7 mo). The rate of CSF flow, optimal position for flow, collection by aspiration compared with gravitation collection, and presence of blood or turbidity were noted. Daily neurologic assessments[8,11] and inspection of the incision sites and port areas for dermal erosion were performed.

Strict aseptic technique was used any time the MILP was accessed. The area was shaved widely and cleaned with chlorhexidine and alcohol. A 24-gauge Huber needle was inserted into the reservoir of the MILP for CSF collection. After sample collection, an amount of sterile isotonic saline (Hospira) equivalent to the volume of CSF removed was flushed into the MILP. When serial samples were taken at close intervals, an abbreviated surgical prep was performed (that is, a single round of chlorohexidine and alcohol). After the last sample of the day or after a monthly maintenance check, triple-antibiotic ointment (bacitracin, neomycin, and polymyxin B, Perrigo, Charlottesville, VA) was placed over the area once the Huber needle was removed.

Figure 1. Insertion and advancement of the catheter through the epidural needle.

Figure 2. Placement of a ligation clip at the distal end of the catheter to prevent any additional loss of CSF.

The criteria for successful functioning of the MILP system were the absence of neurologic sequela and the successful collection, by using aspiration or gravitational flow, of multiple 0.5- to 1.0-mL samples of CSF devoid of contamination by blood over the course of 3 mo.

Results

The MILP system was successfully implanted in all 10 of the macaques. No neurologic deficits, postoperative infection of the surgical site, or skin erosion of the port area developed during the 21.7-mo study duration. Due to clinical illnesses unrelated to this study, 2 macaques were lost from the cohort. The MILP

Figure 3. Confirmation of CSF flow, through the port, after the final skin closure.

system functioned successfully in 7 of the 10 macaques (70%) for 3 mo; within this group, 4 of the 7 cases (57%) retained continuous functionality for 19.2 mo on average. The overall mean duration of functionality for all 10 MILP was 8.5 mo. The MILP functioned intermittently in one macaque (Table 1).

Among the 7 macaques with functionally successful MILP systems, 3 (43%) exhibited positional functionality. For most sampling sessions involving these 3 MILP systems, flow was best established with the animal in the vertical position.

Due to problems with the port, 5 of the 10 cases (50%) required surgical repair during the study duration of 21.7 mo. Specifically, 2 ports were disconnected from the catheter due to tears at the port–catheter stem connection. Degradation of the port septum due to repeat puncture occurred in 2 cases. In one case of port degradation, the port also was transposed (flipped) such that the septum was ventral. In another case the port migrated distally resulting in catheter nonpatency, and the MILP system was removed. In 4 of these 5 cases, surgical replacement and repositioning of the port through a skin incision was successful. The MILP system with the transposed port was successfully replaced; this port continues to be functional but has not yet reached the predetermined 3-mo time point.

The MILP system was unsuccessful in 2 of the 10 macaques (20%) and resulted in a loss of patency at 3 and 16 d (mean, 9.5 d) after implantation. In both cases, the catheter repositioned or replaced during surgery to reestablish CSF flow. However, although the ports were verified as being functional intraoperatively, CSF flow could not be reestablished for more than 1 to 2 d.

Discussion

The penetration of a single agent (or multiple agents in combination therapy) across the blood–brain barrier is the goal of studies assessing the pharmacokinetic and pharmacodynamics of treatments for CNS disease. For these studies, CSF drug levels serve as a surrogate for CNS tissue penetration, and because access to CSF in human studies is limited, this information becomes a crucial component in NHP biomedical translational research. Frequently, the requirement for CSF is further refined to serial sampling, demanding reliable and repeated access to CSF, which needs to be accomplished atraumatically in unanesthetized NHP.

Several current NHP models accomplish this goal of serial sampling of CSF, by using a ventricular reservoir[16] (flexible silastic dome) or intravenous access port[11,13] in either the 4th or lateral ventricle with varying rates of successful establishment and duration.[11] These closed subcutaneous systems all have one attribute in common: surgical invasion of the cranial cavity. In addition, these models share one or more of the following requirements: a stereotaxic unit for placement; MRI-generated coordinates; supplementary materials for attaching the reservoir or port to the skull; and extended and intensive postoperative care; in addition, for many of these models, surgical modification to correct nonpatency is generally not feasible. The development and use of a previous lumbar port system requires a laminectomy for placement[2,5] and, as with the ventricular models previously described, is surgically invasive, postoperatively intensive, and is associated with varied successful establishment and duration.

Although invaluable to their respective research protocol, the development of these CSF collection systems in macaques requires extensive monetary, labor, and time commitments due to the aforementioned characteristics. Where this degree of commitment is not possible due to study requirements or resources, lumbar catheterization is the default serial CSF collection method. This procedure results in an externalized, temporary system for CSF collection that is unreliable and that is not advantageous in a conscious but restrained macaque.

The goal of the current study was to develop a model that bypassed the surgical invasiveness and the monetary, labor, and time commitments of the previously described models, yet still provide a closed, reliable, serial CSF collection system that could be accessed frequently and atraumatically in restrained macaques. The development of the MILP model accomplished this goal by establishing CSF access through a percutaneous stick, without invasion of a body cavity, with minimal surgical intervention required for implantation of the port. The MILP system was implanted without the use of MRI coordinates, a stereotaxic device, or supplementary materials for port attachment. Surgical implantation was successful and well tolerated in all 10 macaques, as evidenced by analgesia maintenance without the use of the prescribed opioids and NSAID, as well as the lack of neurologic deficits, postoperative infection of the surgical site, or skin erosion of the port.

The reliability of MILP model was demonstrated ,with a successful establishment rate of 70% over 3 mo and with continued functionality in 57% of those animals for an average of 19.2 mo. In addition, CSF was reliably obtained in volumes of 0.5 to 1.0 mL, and the MILP system has been used in pharmacokinetic and pharmacodynamic studies in our laboratory that require obtaining CSF samples of 0.3 mL at 0, 5, 15, and 30 min and 1, 2, 3, 4, 6, 8, 10, and 24 h after drug dosage. The samples were collected atraumatically in macaques that were unanesthetized but restrained by using the pole and collar and chairing system.

The 2 problems encountered with the MILP system were nonpatency and port failure. Although the system could be surgically repaired through only a minor skin incision, the repair was only successful whenever the port was replaced. Cases of nonpatency were not resolved successfully through surgery. Only 1 of the 3 cases of intermittent flow was successfully corrected and required replacement of the entire MILP system.

Whether lumbar CSF is an effective surrogate for CNS tissue penetration of an agent in lieu of ventricular CSF must be decided

Table 1. Characteristics of the new CSF sampling device in rhesus macaques

Case no.	Successful implantation?	Functionally successful?	Duration of functionality (d)	Positional flow?
1[a]	Yes	No	3	Not applicable
2	Yes	Yes	686	No
3	Yes	Yes	617	Yes
4	Yes	Yes	612	No
5	Yes	No	35	No
6	Yes	Yes	433	No
7	Yes	No	16	Not applicable
8	Yes	Yes	134	Yes
9	Yes	Yes	98	Yes
10[a]	Yes	Yes	219	No

[a]Animal lost from study due to an unrelated clinical illness.

for the particular study. Some studies have demonstrated a pharmacokinetic difference in drug levels between ventricular and lumbar CSF after intravenous administration.[3,14]

In summary, the MILP model was successfully developed in rhesus macaques and is an appropriate replacement in studies requiring lumbar CSF collection that previously was accomplished by using temporary catheterization or models requiring a laminectomy for placement. In addition, the MILP might be a short-term alternative for the ventricular CSF collection systems in NHP when the source (ventricular or lumbar) of the CSF is not critical.

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