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Use of a Low-concentration Heparin Solution to Extend the Life of Central Venous Catheters in African Green Monkeys (*Chlorocebus aethiops*)

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Normal hematologic values for African green monkeys have been reported, but these results are confounded by the effect of chemical restraint (for example, ketamine), physical restraint, and capture stress. The dual-lumen central venous catheter, jacket, and tether combination we describe here allows intravenous fluid administration and repeated blood sampling without the use of anesthesia or inducing capture-related stress. The use of a low-concentration heparin solution for catheter maintenance significantly increased the mean patency time, compared with a saline-only catheter flush solution. Adding a low-concentration heparin solution creates a suitable system for serial blood collection in the African green monkey for as long as 25 d.

Researchers that use animals have always struggled to identify improved methods that minimize the effects of experimental manipulation on the animal's physiology. Acquiring blood samples from nonhuman primates historically has required the use of chemical or physical restraint (with or without acclimation) and, more recently, extended periods of training to allow 'voluntary' sampling of unsedated animals. Normal hematologic values for African green monkeys have been reported,[3,7,10] but these results are confounded by the effect of chemical restraint, physical restraint, capture stress, or a combination of these stressors (for example, animals in outdoor enclosures must be captured and restrained to administer a sedative). For protocols requiring repeated sampling, habituation to such repetition could affect the interpretation of both research and clinical data. Some researchers suggest that "repeated bleeding with ketamine anesthesia is initially not stressful but becomes so, whereas repeated bleeding in tame animals without anesthesia is initially stressful but becomes less so with each bleeding."[10]

In the present study, our intent was to use a system that would allow intravenous administration of a test substance and repeated blood sampling of African green monkeys without anesthesia or inducing capture-related stress. By eliminating these 2 stressors, we prevented their effects on body temperature, activity, and appetite, which are often key factors in the clinical assessment of animals used in infectious disease research. Early forms of a jacket and tether system for nonhuman primates were developed and used extensively at our institution to address problems associated with chair restraint.[1,5] For the current project, we chose a dual-lumen central venous catheter, jacket, and tether combination that was in use in a National Institutes of Health laboratory. Our veterinary and technical staff received training at that facility in the surgical technique and the extensive catheter care procedures. Our challenge was to modify this system to allow repeated blood sampling over a period of at least 24 d postsurgery and to minimize the effects of potential catheter-related factors on the experimental results. Introducing a foreign body into an experimental animal is not a benign process, and anyone considering the use of an indwelling catheter should carefully weigh the value of the potential results against the very real dangers associated with catheter use. Meticulous aseptic technique is required to avoid catheter-related infection and septicemia. In 1 study, catheter-tract infections were diagnosed in 30.2% of catheterized rhesus macaques.[9] In another, rhesus macaques developed catheter-related septicemia, and antibiotic therapy and catheter removal was required to resolve the bacteremia.[2]

Materials and Methods

Animals. We selected 25 (13 male and 12 female; weight, 3.1 to 6.2 kg) African green monkeys (*Chlorocebus aethiops*, formerly *Cercopithicus aethiops*, originating from St Kitts) from the in-house colony for this study. All animals were clinically normal on physical examination and were seronegative for measles virus, *cercopithecine herpesvirus* 2, simian immunodeficiency virus, and simian T-cell leukemia virus. Four animals tested positive for rotavirus (SA11) antibody. No intestinal parasites were detected upon fecal examination. All of the animals were housed in 4.5-ft^2, 4 cage-per-rack units (Allentown Caging Equipment, Allentown, NJ), and environmental conditions were maintained as recommended in the *Guide for the Care and Use of Laboratory Animals* [6] (temperature, 17.8 to 29.9 °C; humidity, 30% to 70%; and 12:12-h light:dark cycle). Animals were fed a standard primate diet (no. 8714, Harlan Teklad, Madison, WI) supplemented with fruit and other food treats. Fresh water chlorinated at the municipal level and filtered through a water system (Edstrom Industries, Waterford, WI) in the facility was provided ad libitum. Environmental enrichment was provided (Hanging Ball, Bio-Serv, Frenchtown, NJ), and cages were arranged so that the animals were facing each other across the room.

The described procedures were performed as part of an animal research protocol reviewed and approved by our facility's laboratory animal care and use committee. This research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to the *Guide for the Care and Use of Laboratory Animals*.[6] The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

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Experimental groups. The work described here was conducted to support 2 protocols at our institution that required intravenous administration of antibiotics. For each protocol, animals were randomized into 2 experimental groups of 6 animals each. An additional animal was added to the first experiment to replace an animal that experienced catheter failure before the start of the parent experiment. The first group received saline infusion (0.9% Sodium Chloride Injection USP, Baxter Healthcare Corporation, Deerfield, IL) to maintain the catheters. The blood sampling port of the remaining 3 groups was maintained by a 1-U/ml heparinized saline lock solution (Heparin Sodium Injection USP, Baxter Healthcare Corporation, in 0.9% Sodium Chloride Injection USP, Baxter Healthcare Corporation). This lock solution formulation had been used with success at our facility in the past,[5] but it was not compared with saline or other locking solutions at the time. Overall animal numbers (n = 25) were selected to support the needs of the parent experiments; group size (n = 6) was selected to accommodate the personnel necessary and available to conduct these experiments.

Presurgical preparation. Each animal was sedated with a ketamine hydrochloride (9 mg/kg) and acepromazine (0.1 mg/kg) combination (0.1 ml/kg intramuscularly), intubated, and maintained with 1.0% to 2.5% isofluorane anesthesia. Surgical sites were prepared by clipping the hair in wide margins; sites were scrubbed and rinsed 3 times with alternating povidone-iodine solution and 70% alcohol. Buprenorphine hydrochloride (0.01 mg/kg), glycopyrrolate (0.06 mg/kg), and cefazolin (20 mg/kg) were administered intramuscularly to each animal immediately before moving it into the surgery suite. A final surgical scrub and rinse with povidone-iodine solution and 70% alcohol was performed at the surgery table.

Surgery. Two surgical procedures were performed on each animal. The first was placing a temperature- and activity-monitoring telemetry device (Physiotel TA-D70 Large Animal Transmitter, Data Sciences International, St Paul, MN). A paramedian incision was made over the left abdomen. Blunt dissection was used to form a subcutaneous pocket. The telemetry device was inserted, and the skin was closed in a simple continuous subcuticular pattern (3-0 Vicryl, Ethicon, Somerville, NJ). Each animal recovered in its own cage and was allowed a minimum of 14 d to recuperate before the catheter surgery.

To place the catheter (Hickman 7 French Dual-Lumen CV Catheter, Bard Access Systems, Salt Lake City, UT), an incision was made on the right side of the neck, exposing the internal jugular vein. Bupivicaine hydrochloride (0.3 ml, 0.25% solution) was instilled into the incision site to minimize vasoconstriction associated with manipulation of the jugular vein. Blunt dissection was used to free 2 to 3 cm of the internal jugular vein from the remainder of the vascular bundle. A trocar was used to create a subcutaneous tunnel from the cervical surgery site, over the right shoulder, to a point in the center of the back, where a small incision was made to expose the trocar tip. The catheter was attached to the trocar and retracted into the cervical surgery site until the cuff was approximately 1 cm under the skin. The catheter then was trimmed, leaving 10 to 12 cm exposed at the jugular cut-down site. The vein was incised, and the free end of the catheter was inserted into the vessel and advanced to position the catheter tip at the junction of the superior vena cava and the right atrium of the heart. The catheter was inserted by hand to avoid increased thrombogenicity resulting from the use of introducers and protective sleeves.[4] The catheter was secured in place with 2 cerclage sutures (2-0 silk, Ethicon, Somerville, NJ) placed above and below the jugular insertion site. Catheter function was verified by aspirating 5 ml of blood and then flushing with 10 ml of normal saline, followed by 3 ml of 1-U/ml heparin in saline. The neck incision was closed in a simple continuous subcuticular pattern (4-0 Vicryl, Ethicon, Somerville, NJ), leaving the remainder of the catheter in the subcutaneous space to allow free movement of the head and neck and to prevent positional tension on the catheter. After extubation, the animals were placed in nonhuman primate jackets (Large Animal Jacket, Lomir Biomedical, Malone, NY), returned to their cages, and attached to a tether system (Large Animal Tether, 24 in., Lomir Biomedical) and syringe pump. Catheter surgeries were conducted over 2 d, with 3 animals each day.

Postsurgical care. After both surgical procedures, each animal received buprenorphine hydrochloride (0.01 mg/kg intramuscularly) and cefazolin (20 mg/kg intramuscularly) twice daily for 2 d. All animals were observed twice daily for postsurgical complications. No postsurgical complications were noted during the study.

Catheter maintenance. The dual-lumen catheter selected for this study had a white port for infusion and a red port for blood sampling. In the saline group, a constant rate of 2 ml/h was used for each catheter port, for a total of 4 ml/h administered to each animal for the duration of the study. During blood sampling, the fluid pump was stopped, the residual fluid in the tether line (approximately 2 ml) was aspirated, a blood sample was collected, the line was flushed with 10 ml of saline, and the syringe pump was restarted. For the heparin group, a constant rate of 2 ml/h was used to administer saline through the white catheter port only. During blood sampling, the syringe pump was stopped, the red port of the catheter was uncapped, the line was flushed with 10 ml of saline, the residual saline in the line (approximately 2 ml) was aspirated, a blood sample was collected, the line was flushed with 10 ml of saline and then flushed with 3 ml of a 1-U/ml heparin saline solution, the red port was recapped, and the syringe pump was restarted.

Blood sampling. A single sample (2 ml) was drawn at surgery to verify the patency of the catheter. An additional sample (2 ml) was taken for baseline data at 6 to 7 d postsurgery. Routine blood sampling (2 to 4 ml, depending on tests to be performed) started 9 to 10 d after the catheter surgery and was repeated every 48 h for 14 d. After the sampling period, the catheters were aspirated and flushed every other day to assess patency, but no blood was withdrawn for analysis. The date that blood could no longer be aspirated from the red port of the catheter was recorded as the failure point.

Statistical analysis. Animals that were eliminated from the studies for factors other than catheter failure were excluded from analysis. Patency survival curves were calculated by using the Kaplan-Meier method. Survival curves were compared by using log-rank tests. Mean time-to-failure was compared using a Student *t* test. The effects of sex and study as covariates were examined before pooling data across studies. Analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC, 2003).

Results

In the saline group, duration of patency ranged from 3 to 22 d (average, 12.2 d; median, 12 d; n = 5). One animal was excluded for factors other than catheter failure. In the heparin group, duration of patency ranged from 18 to 38 d (average, 25.8 d; median, 27 d; n = 14). Five animals were eliminated for factors other than catheter failure.

Analysis of rate of patency over time. In order to pool animals with heparin-infused catheters across studies, differences between studies first were examined. Results of the Kaplan-Meier analysis comparing animals with heparin-locked catheters from the first study with those from the second study showed that rate of catheter patency did not differ significantly ($P = 0.5001$).

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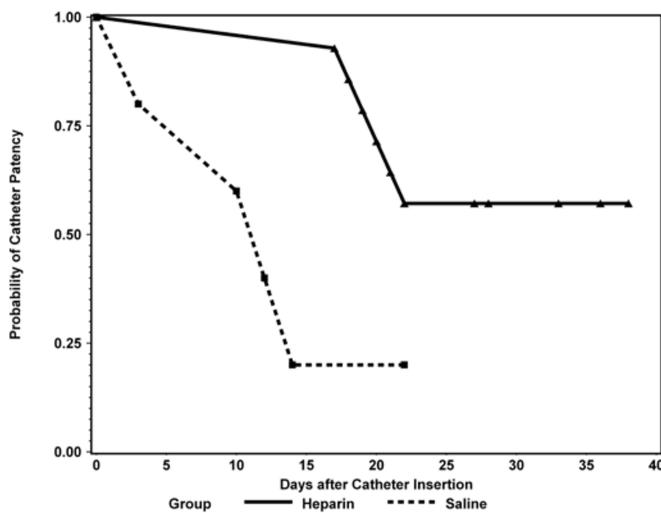

Figure 1. Probability of catheter patency after catheter insertion.

In addition, results of a Kaplan-Meier analysis showed that there was not a significant difference ($P = 0.2031$) between sexes in rate of catheter patency. Because no differences were found between sexes or studies, further analysis of catheter patency was conducted without including these covariates. Results of the Kaplan-Meier analysis comparing catheter treatment (Figure 1) showed there was a significant ($P = 0.0085$) difference between groups in rate of catheter patency. In the saline group, the probability of the catheter remaining patent decreased steadily after insertion. In the heparin group, the probability of the catheter remaining patent stayed at 100% until 15 d after insertion and did not fall below 50% over the course of the study. Because of small sample sizes, the median patency time could not be calculated; however, mean patency time was calculated. The duration of patency (mean ± standard error) for the saline group (n = 5) was 12.2 ± 3.2 d compared with 30.1 ± 2.7 d for the heparin group (n = 14).

Analysis of mean time to failure. Results of a *t* test comparing mean time to catheter failure showed a significant ($P = 0.0018$) difference between the heparin and saline groups. The heparin group had a longer mean time to catheter failure than did the saline group. The mean failure time for the saline group (n = 4) was 9.8 ± 2.4 d compared with 19.5 ± 0.7 d for the heparin group (n = 6).

Discussion

The use of a 1-U/ml heparin solution significantly increased the useable lifespan of central venous catheters in African green monkeys, compared with saline infusion. Of the catheters that failed during the sampling period, those maintained with heparin lasted twice as long as those with only a saline infusion. In the saline group, only 1 of 5 catheters remained patent for the entire 14-d sampling period, while 11 of 14 catheters in the heparin group remained patent throughout the sampling period. The statistical power of this result would have been improved had the saline group been larger, but we made the change to a heparin solution in response to poor duration of catheter patency in the first group.

Our objective was to maintain catheter patency throughout the postsurgical period (9 to 10 d) and sampling period (14 d). Long-term catheterization (average, 274 d) of cynomolgus monkeys has been reported,[8] but the locking solution used immediately postoperatively was 1000 U/ml heparin. Catheters then were flushed weekly and locked with a sodium heparin (900 U/ml)–gentamicin (5 mg/ml) solution. In a study using rhesus macaques, the mean lifespan of all catheters was 338 d; catheters were flushed twice daily with approximately 2 ml of sterile heparinized saline (100 U/ml).[9] We determined that neither of these solutions were acceptable in our research context.

Although the use of a low-concentration heparin solution met our immediate needs for our project, we identified several modifications that may extend the patency period. In future experiments, we intend to flush the catheter daily rather than every other day. We also will acclimate the animals to the jackets and tethers for 7 to 10 d before the catheter surgery. Shifting the acclimation period to before the surgery will decrease the time between the catheter surgery and the start of an experiment from 6 to 7 d to 2 to 3 d. Increasing the concentration of the heparin solution also may extend the life of the catheter, but further work is necessary to determine the effect, if any, of chronic low-dose heparin administration on the hemogram of the African green monkey. It is also possible that increasing the infusion flow rate of the saline system would provide sufficient catheter lifespan for serial sampling over a longer period of time. This saline protocol would be the approach of choice if coagulation parameters were to be measured. We believe that the methods that we have adopted will prove to be a suitable system for serial measurement of hematologic values in African green monkeys, free from the effects of anesthesia and capture stress.

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