

Patency of Jugular Vein Catheters in CD-1[®] IGS Mice: Evaluation of Three Catheter Maintenance Schedules in Standardly Externalized Catheter and Transcutaneous Buttons

Sera Mallette, Tessa Murray, Venkateswarlu Karicheti, Yiyang Luo, Allison Williams, Dawn Decker, Tiffany A Weller
Charles River Laboratories, 251 Ballardvale Street, Wilmington, MA 01887

1 ABSTRACT

Pharmacokinetic studies in mice are conducted using chronically implanted jugular vein catheters (JVCs) that allow central venous access in conscious animals for repeated blood sampling and dosing; however, maintaining continuous patency sets practical limits on its uses. We conducted a study investigating three different catheter maintenance schedules for mice with JVCs with standard externalization (STNRD) compared to a transcutaneous button (BUTTON). Seventy-two adult male CD-1[®] IGS mice [CrI:CD1(ICR)], 27 - 30 g, were randomly allocated into six groups (n = 10 each) based on catheter flushing frequency, i.e., once every 3 (q. 3), 5 (q. 5), or 7 (q. 7) days, starting 3 - 4 days after surgery. For groups 1 - 3, the catheter was sealed with a metal plug and the extravascular portion was extended subcutaneously, exiting at the interscapular region. In groups 4 - 6, the catheter was connected to a transcutaneous button located in the same interscapular region. A locking solution of heparinized (500 IU/mL) 50% dextrose was administered and used for the duration of the study. Animals were shipped to another Charles River site for patency checks to mimic a standard customer order. The catheter was considered patent if blood was successfully withdrawn. Animals were clinically healthy throughout the study. Catheter patency rates were 100% in all animals on the first assessment. At approximately 2 weeks post-surgery (10 - 14 days), 100% of q. 3 and q.7 catheters with the BUTTON remained patent, and 90% of the q.5 were patent. The STNRD dropped to 20%, 30%, and 50% patency for q.3, q.5, and q.7, respectively. At conclusion of the study at day 45 - 46, 10% (1 animal) of the STNRD remained patent for blood collection from each group; however, 80%, 40%, and 70% remained patent for the BUTTON. In summary, 90% of catheters using the BUTTON remained patent two weeks postoperatively, irrespective of the catheter maintenance schedule. The use of transcutaneous buttons kept the catheters patent for up to 5 times longer than the standard externalization.

2 INTRODUCTION

Pharmacokinetic studies are used to determine the concentrations of test compounds and other therapeutic area/drug discovery target-specific analytes in the blood at specific time points.

Refinements have been made to decrease the volume of blood needed for *in vitro* analysis, allowing for a single animal to be sampled for the duration of the study. This has provided more consistent data as well as reduced the number of animals needed for study.

Blood can be collected from animals via various routes, e.g., tail vein, submandibular, and retro-orbital. However, these methods have limitations, e.g., animal welfare and number of time points, volume of blood that can be collected.

The use of vascular catheterized animals allows for multiple blood collections to occur without venipuncture as well as allowing collections to occur from a more centralized vessel. Patency of the cannula has been a limiting factor when used in mice.

We explored two different externalization techniques of the catheterization over three different catheter maintenance schedules to determine the best model to use for repeat blood sampling.

3 MATERIALS AND METHODS

Animals

72 male CD-1[®] IGS mice [CrI:CD1(ICR); Charles River Laboratories, Kingston, NY], of VAF/Plus[®] health status, weighing between 25 - 30 grams, were surgically instrumented. Following surgery, mice were transported in divided, filtered shipping containers to Charles River Laboratories, Shrewsbury, MA for evaluation of patency. In Shrewsbury, mice were singly housed in polycarbonate cages and maintained at 21 ± 2 °C with relative humidity of 30 - 70% and a 12:12 hour dark:light cycle. Commercially produced feed and water were provided *ad libitum*. All conditions of animal preparation and use were in accordance with recommendations set forth in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011).

Surgical Procedures

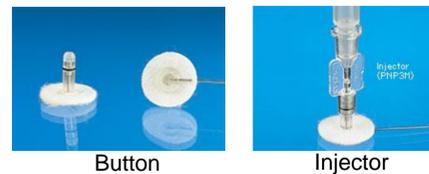
Anesthesia/Analgesia: Ketamine (75 mg/kg) and xylazine (14.5 mg/kg) injected intraperitoneally, buprenorphine (0.05 mg/kg) injected subcutaneously. Animals were allowed to recover in a cage placed on a heating pad. **Implantation of Catheter:** 36 animals were implanted with a jugular vein catheter (JVC) using standard externalization (STNRD). Catheter was secured with wound clips and ventral skin incision closed with suture in a subcuticular pattern. 36 animals were implanted with a JVC attached to a transcutaneous button (BUTTON), Instech model # VAB62BS/22. Button was secured with suture.

Patency Testing (Flushing)

Testing Solution: Sterile saline.

Locking Solution: Heparinized (500 IU/mL) 50% dextrose.

Method: Animals were manually restrained and catheters accessed using blunt tip needles for STNRD catheters and an injector (Instech model # PNP3M) for BUTTONS.



Necropsy

At the end of the study animals were euthanized and gross necropsy was conducted to identify causes of patency loss.

4 EXPERIMENTAL DESIGN

The 72 mice were allocated into 6 groups consisting of 10 mice each (and 12 spares) for flushing once every 3, 5, or 7 days for each exteriorization type, starting 3 - 4 days after surgery. Spares were not used on study.

Patency Categorization:

- Fully Patent (FP): Successful blood withdrawal on first attempt
- Patent on Flush (PF): Successful blood withdrawal after infusion of saline
- Partially Patent (PP): Unsuccessful blood withdrawal but patent for infusion
- Non-Patent (NP): Unsuccessful blood withdrawal and infusion

Group	Catheter Instrumentation	No. of Males	Catheter Maintenance Schedule
1	Jugular Vein Catheter (STNRD)	10	Once every 3 days
2		10	Once every 5 days
3		10	Once every 7 days
4	Jugular Vein Catheter and Transcutaneous Button (BUTTON)	10	Once every 3 days
5		10	Once every 5 days
6		10	Once every 7 days

5 RESULTS

STNRD - Flushing every 3 days

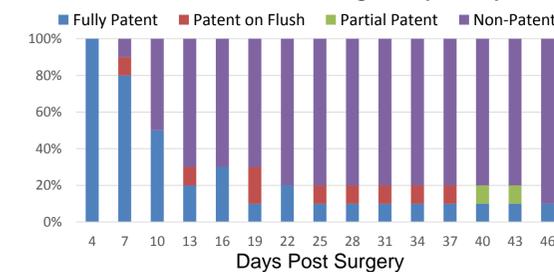


Figure 1. Patency of standard JVC flushed every three days. Data is expressed as percent patent. Causes of loss of patency: Unknown = 4, Clot in line = 1, Catheter out of vessel = 1, Animal health = 1.

STNRD - Flushing every 5 days

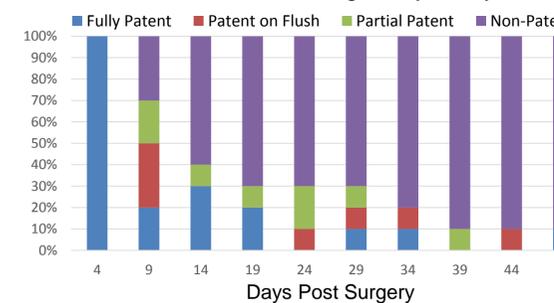


Figure 3. Patency of standard JVC flushed every five days. Data is expressed as percent patent. Causes of loss of patency: Unknown = 3, Catheter out of vessel = 2, Animal health = 4.

STNRD - Flushing every 7 days

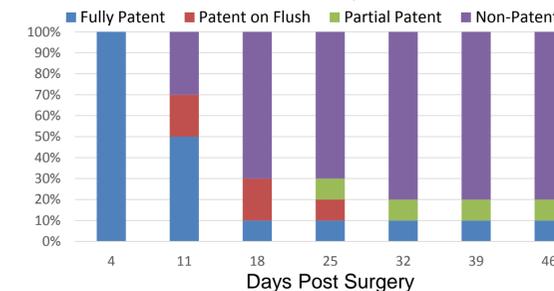


Figure 5. Patency of standard JVC flushed every seven days. Data is expressed as percent patent. Causes of loss of patency: Unknown = 7, Catheter out of vessel = 1.

BUTTON - Flushing every 3 days

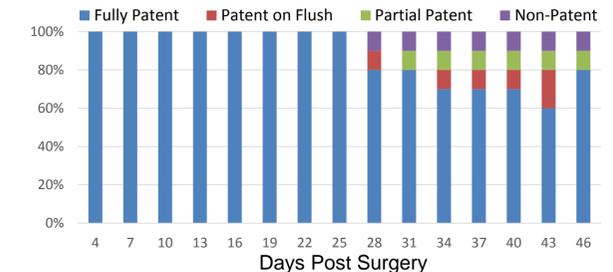


Figure 2. Patency of button JVC flushed every three days. Data expressed as percent patent. Cause of loss of patency: Disconnected from button = 1.

BUTTON - Flushing every 5 days

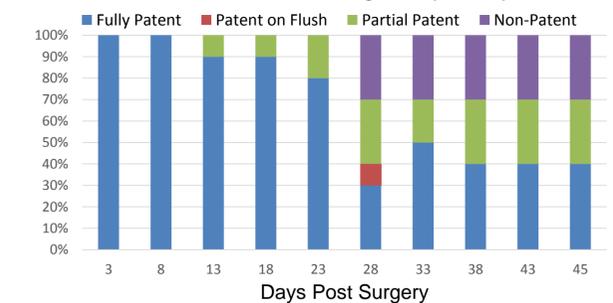


Figure 4. Patency of button JVC flushed every five days. Data expressed as percent patent. Causes of loss of patency: Disconnected from button = 1, Unknown = 1, Animal health = 1.

BUTTON - Flushing every 7 days

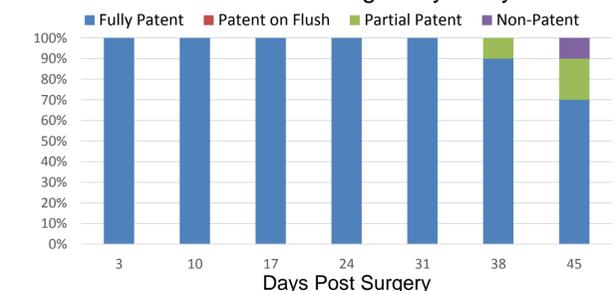


Figure 6. Patency of button JVC flushed every seven days. Data expressed as percent patent. Cause of loss of patency: Disconnected from button = 1.

6 DISCUSSION

The use of transcutaneous buttons kept the catheters patent for up to 5 times longer than the standard externalization. Irrespective of the catheter maintenance schedule, 90% of catheters using the BUTTON remained patent two weeks postoperatively. The standard PK study runs 24 hours to 1 week in duration, allowing the BUTTON catheter to be an option for blood collection and a refinement to these early discovery studies.