Refinements to Intermittent IV Infusion Procedures in C57BL/6 Mice to Achieve Study Endpoints and Improve Animal Welfare

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1 ABSTRACT

Intermittent intravenous (IV) infusion in mice is challenging. The traditional method for IV dosing is via the tail vein. This requires animal restraint and the use of an extension line with a needle attached to a syringe. Restraining mice can cause stress and potential injury. Catheters are technically challenging to place in the tail vein and may damage the internal lining of the vessel. Alternatively, mice are restrained for dosing, so surgically implanted intravenous catheters. Surfacing is impractical for the typical dose regimen for an intermittent IV infusion study - once weekly for up to 35 minutes for 6 weeks as mice remain attached to the tether system and individually housed for the duration of study. SM and SF C57BL/6 mice were surgically implanted with jugular vein catheters attached to a transcutaneous button at a Charles River Research Model Service site and were transported to the study site. The mice were dosed with 0.5% sterile saline twice weekly at 2.5 mL/kg/mouse for 6 minutes via an extension set attached to a button injector. Animals were removed from their cages, placed into a dosing cage, and were able to move freely. Various sizes of cages, with and without lid and enrichment, were tested. Ports were inoculated with 5.4cc, heparinized saline following each dose. All mice were successfully dosed for 6 weeks with no effects on body weights or clinical condition. Allowing mice to roam around a cage was an improvement to animal welfare and did not require restraint or tethering to deliver the dose successfully.

2 METHODS

Five male and 5 female C57BL/6 mice were ordered for a method development study to determine the feasibility of allowing mice to roam freely around a cage during intermittent infusion dosing. The mice were surgically implanted with jugular vein catheters at Charles River Research Model Service. The rounded polyurethane catheter was bonded to a transcutaneous button which was placed in the scapular area and protected by a sterile metal cap. At the start of study, the mice were approximately 9 weeks of age and weighed 20 – 25 grams. The mice were identified by microchip and single housed in polypropylene cages with paper bedding material. Animals had access to certified rodent pellets, water and enrichment enrichment ad libitum except during dosing. During the first dose, mice were housed in mouse shoe box cages with lids. Food enrichment and nesting material was provided. For all remaining doses, mice were housed in empty rat shoe box cage without lids (Figure 1).

Mice were dosed twice weekly with 0.5% sterile saline at a rate of 2.5 mL/kg/mouse for 6 minutes. The protective cap was removed using a tool that detached magnetically to the base of the transcutaneous button. The button extension line was sterilized with 70% isopropanol alcohol prior to attaching with a syringe attached to a button-injector and an attempt was made to draw off the heparinized saline lock. The port was then flushed with sterile saline. The pre-filled extension line and button injector were attached to the button and the mouse was placed in the dosing cage to freely roam during the infusion (Figure 2). Body weights and detailed observations were collected once weekly, daily observations were collected during the dosing period. Following the 6-week dosing period, an attempt was made to prolong the infusion period to 35 minutes using the dosing equipment described above as well as an extension set with a built-in injector which is lighter weight (Figure 3). Animals were then transferred to the training colony.

Figure 1 – Dosing set up – rat caging and infusion pumps.

Figure 2 – Cage exploration behavior.

3 RESULTS

After six weeks of twice weekly 6-minute infusions, all mice were patient for dosing. A rat shoebox cage proved to be the best for dosing as there was no lid to interfere with the extension line and the height prevented animal escape. The mice received food enrichment and nesting material, so this was discontinued after the first week. Mice spent the entire dosing period exploring the cage (Figure 2) and did not disturb the extension line or injector. Body weights and body weight gain (Table 1) were not affected by the surgical implant or the dosing procedures. The surgical site for the transcutaneous buttons healed well, and the animals were observed to be clinically normal. While blood sampling was not part of this study, we were able to collect blood from the tail without any interference from the transcutaneous button (Figure 4). Dosing periods greater than 10 minutes were not successful; once the cage exploration behavior ended, the mice attempted to disconnect the extension line with injector (50% were successful) and bit through the lighter weight injector.

4 CONCLUSIONS

- The use of jugular vein catheters attached to transcutaneous buttons was successful for short-term intermittent intravenous infusion dosing of non-restrained mice twice weekly for 6 weeks.
- Animal welfare was improved over traditional dosing methods as animals were able to ambulate during dosing and there was no need for restraint, continuous tethering, or repeat catheter placement in the tail vein.
- The surgical and dosing procedures had no effect on body weights, body weight gains, or clinical observations.
- The protective metal cap on the transcutaneous button allowed for social housing of mice without damage to the transcutaneous buttons.
- Blood collection from the tail vein is possible with an appropriate restraint device to accommodate the transcutaneous button.
- Intermittent IV infusions greater than 10 minutes was not successful. Once cage exploration behavior ended, mice attempted to remove the injector and extension line.

Table 1 – Surgical and dosing procedures had no effects on body weights.

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<tr>
<th>Average Bodyweight</th>
<th>Male BW (g)</th>
<th>Female BW (g)</th>
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<td>22.54 ± 0.3</td>
<td>22.96 ± 0.3</td>
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Figure 3 – Light weight extension line.

Figure 4 – Blood collection in clamshell restrainer.

Figure 5 – Surgical and dosing procedures had no effects on body weights.