Twenty-eight Day Continuous Intravenous Infusion in Mice: Use of Externalized Magnetic Ports and Tethers to Achieve Study Endpoints and Enhance Animal Welfare

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1 ABSTRACT

Continuous intravenous (IV) infusion in mice presents many challenges. The model requires intricate surgery and catheter size is limited due to vessel size. In addition, traditional tether security devices such as jackets, harnesses or tail-cuffs need to be continually checked and adjusted for proper fit. Improper fit of jackets or harnesses can lead to irritation, skin lesions, and swelling of the anterior body. Tail-cuffs can cause swelling and lesions where they are secured with steel suture. If the devices are too loose, the mouse can escape and destroy the exteriorized catheter, leading to patency issues and potential infection. A validation study was conducted comparing continuous infusion of 0.9% Sterile Saline for Injection (saline) and 20% hydroxypropyl-beta-cyclodextrin (HBC) in sterile water. Twenty-five CD1 male mice implanted with jugular catheters attached to an externalized port were purchased. Male mice were used since gender differences were not expected. The ports of 20 mice (10 per group) were connected to a magnetic tether/swivel assembly mounted to a counterbalance arm on the cage lid. The animals were infused at a rate of 2 mL/kg/hr for 27 or 28 consecutive days. Due to toxicity in 5 animals and a blocked catheter in 3 animals between days 5-10 in the 20% HBC group, the surviving and replacement animals were placed on a saline dosing holiday followed by dosing the remaining 10 or 17 days with 10% HBC. Animals were observed daily, and body weights, food weights and detailed physicals were collected weekly. Blood was collected for hematology and serum chemistry at the time of scheduled necropsy and histopathology was conducted on select tissues. All animals were patent for 27 or 28 days of dosing; 90% and 57% of the saline / HBC groups (respectively) were patent for blood draw at necropsy. There were no clinical, gross necropsy or histological signs of infection and white blood cell counts were normal. When both groups were compared to the historical reference, mean AST, ALP, SDH, and A/G ratio were slightly higher while mean globulin was slightly lower. The tether/port connection remained intact for all animals throughout the study and only one animal had a skin scab around the port beginning on day 14. The use of an externalized magnetic port and tether system proved successful in this 28-day continuous IV infusion study and was an improvement in animal welfare when compared to the use of jackets, harnesses, or tail-cuffs.

2 METHODS

To evaluate the feasibility of conducting a 28-day continuous intravenous infusion study in mice equipped with jugular vein catheters and externalized magnetic ports, a method development study was designed. Two commonly used vehicles for infusion studies were tested - 0.9% Sterile Saline for Injection (saline) and hydroxy-beta-cyclodextrin (HBC). Twenty-five male CD1 mice were implanted by the animal vendor with polyurethane catheters placed in the jugular vein, advanced to the atrium, and exteriorized to an external magnetic port. The rounded tip catheter was previously bonded to the subcutaneous pin of the port by the port manufacturer. Animals were identified by microchip and single housed in polycarbonate caging. The animals were housed in accordance with the *Guide for the Care and Use of Laboratory Animals*. The animals were 8 weeks old at the start of study and weighed 28.0-38.3 grams. Following a 7-day acclimation period, the ports of 20 animals (10 per group) were connected to a magnetic tether/swivel assembly mounted to a counterbalance arm on the cage lid (see Figure 1). The cages were placed on custom shelving to allow access to the automatic watering system of specially designed rat infusion banks[1] (see Figure 2). Group 1 animals were dosed with 0.9% saline and group 2 animals were dosed with 20% HBC at the start of study. Due to unexpected mortality in group 2, animals were put on a 12-day dosing holiday beginning on day 10 and administered saline only. Dosing for group 2 was subsequently completed with 10% HBC. Including replacement animals, 14 total mice were assigned to group 2. All surviving animals were infused at a rate of 2 mL/kg/hr for 27 or 28 consecutive days. Animals were observed daily, and body weights, food weights and detailed physicals were collected weekly. Blood was collected from the retro-orbital sinus of anesthetized mice for hematology and from the posterior vena cava for serum chemistry at the time of scheduled necropsy. A complete set of tissues were collected for each animal at necropsy. Histopathology was conducted on the heart, kidney, lung, infusion site and gross lesions.

Figure 1

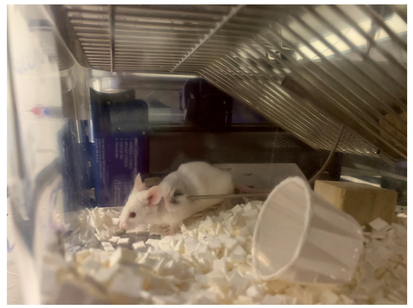

Table 1

Tissue	Early Death/Termination	Scheduled Termination	
	Group 2 (20% HBC)	Group 1 (0.9% Saline)	Group 2 (10% HBC)#
Heart: Thrombus*	**3 / 7**	**3 / 10**	**3 / 7**
Mild	0	3	2
Moderate	2	0	1
Marked	1	0	0
Lung: Thrombus, Arterial*	**3 / 7**	**9 / 10**	**5 / 7**
Minimal	2	4	1
Mild	1	5	1
Moderate	0	0	2
Marked	0	0	1

* severity based on a five-level scale of minimal, mild, moderate, marked and severe
\# one animal was found dead just prior to scheduled termination

Figure 2

3 RESULTS

Between study day 5 and study day 10, several mice in the 20% hydroxypropyl-beta-cyclodextrin (HBC) group had body weight loss and decreased activity; 5 died or were euthanized. Additionally, 3 animals had blocked catheters due to the viscosity of the 20% HBC. The surviving animals in this group were placed on a 12-day dosing holiday and were continuously infused with 0.9% saline at the dosing rate of 2 mL/kg/hr. The two surviving HBC animals and five replacement mice were subsequently returned to dosing with 10% HBC following the dosing holiday. The study was extended for the replacement animals, so all animals were continuously infused for 27 or 28 consecutive days. There were no effects on body weights, body weight gains or clinical observations in the saline group. For group 2 mice, once the dosing holiday began and continuing through the 10% HBC dosing, body weights, body weight gains and clinical observations were comparable to 0.9% saline group. The only infusion equipment related clinical finding was mild scabbing around the port site for one HBC animal beginning on day 14. Hematology parameters were normal for both the 0.9% saline and 10% HBC groups when compared to historical control data. Serum chemistry results showed a slight increase in mean AST, ALP, SDH and A/G ratio and a slight decrease in mean globulin when compared to the historical reference ranges. There were no gross necropsy findings noted for the saline or 10% HBC at the scheduled necropsy. Gross necropsy findings associated with 20% HBC administration noted in mice that died or were euthanized in extremis included a thymus mass in 2 mice, a thymus adhesion in 1 mouse, and a heart mass in 1 mouse. Notable histology findings for animals that died, were euthanized in extremis or at scheduled necropsy included thrombi in the heart and lungs of the 0.9% saline and HBC groups (Table 1). Inflammation and infiltrates were more common and more severe in the HBC group. The inflammation in the heart was more severe in animals euthanized in extremis or found dead. Inflammation with or without fibrosis was noted at the infusion site of HBC animals. Findings of thrombi with related inflammatory lesions are often noted in the lung and heart as secondary lesions related to post-surgical / traumatic conditions and thus this finding did not impact the study objectives.[2] HBC-related foamy macrophage aggregates were noted in the lung and proximal tubular vacuolation was noted in the kidneys of HBC infused mice.

4 CONCLUSION

- 20% hydroxypropyl-beta-cyclodextrin delivered via continuous intravenous infusion was not tolerated causing catheter blockages, body weight losses, decreased activity, and mortality. 10% hydroxypropyl-beta-cyclodextrin proved to be a viable vehicle for continuous intravenous infusion as did 0.9% Sterile Saline for Injection.
- At the time of necropsy all animals were patent for infusion, and blood could be withdrawn from the port in 90% of the saline group.
- There were no clinical, gross necropsy, or histological signs of infection and white blood cell counts were normal.
- Tethering of mice with a magnetic connection improved animal welfare compared to jackets, harnesses or tail cuffs. These traditional tether security devices are often associated with swelling, irritation, and skin lesions. Only one animal on this study had an infusion equipment related observation of mild scabbing around the port site.
- The use of externalized ports with a magnetic tether/swivel assembly allowed for successful delivery of two vehicles via continuous intravenous infusion over a 28 day period.

[1] A. Evans, D. Cedeno Sanmartin, M. Stamen, T. Gleason. The Design and Refinement of a Solid-Bottom Caging Rat Infusion Bank Model. *American Association for Laboratory Animal Science Meeting* 2017.

[2] Weber, K., Mowat, V., Hartmann, E., Razinger, T., Chevalier, H. J., Blumbach, K., Green, O. P., Kaiser, S., Corney, S., Jackson, A., & Casadesus, A. (2011). Pathology in Continuous Infusion Studies in Rodents and Non-Rodents and ITO (Infusion Technology Organisation)-Recommended Protocol for Tissue Sampling and Terminology for Procedure-Related Lesions. *Journal of toxicologic pathology*, *24*(2), 113–124. https://doi.org/10.1293/tox.24.113

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