Serial blood sampling and comparison of three 91-day continuous intravenous infusion models in the rat in safety assessment studies

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Background and objectives

The rat is a commonly used animal model for preclinical continuous intravenous infusion. Many continuous intravenous infusion models have been developed. Due to the possible irritant properties of some test articles, the favoured catheterisation site has become the femoral vein.

The exit site of the catheter is the main difference between the various continuous intravenous infusion models. The catheter may exit via the tail using a tail cuff (TC) and spring device, which is held in place with sterile surgical wire (Figure 1), via the scapular region and secured by a jacket or vascular access harness (VAH) (Figure 2) or via a vascular access button (VAB) (Figure 3).

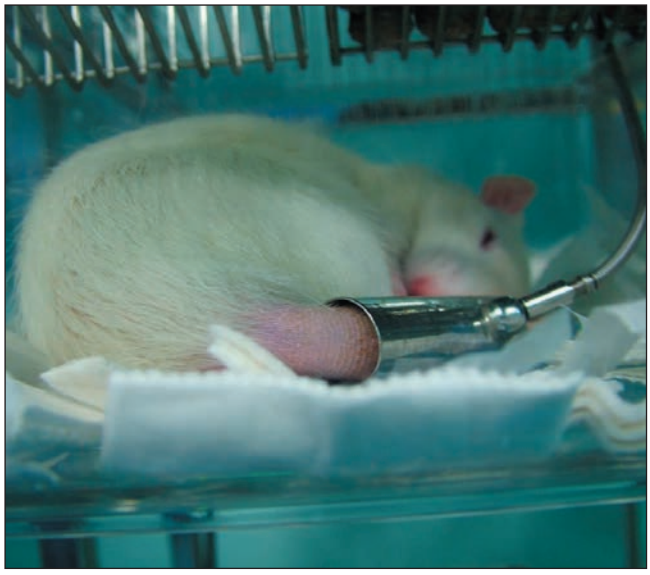

Figure 1. Tail cuff (TC).

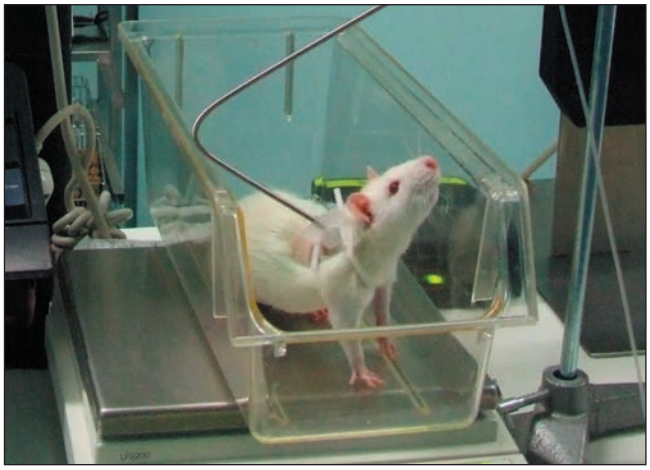

Figure 2. Vascular access harness (VAH).

Figure 3. Vascular access button (VAB) connected to harness.

The objectives for this 13-week continuous infusion period were to determine (1) which exteriorisation methodology was most reliable and (2) which exteriorisation method facilitated successful blood sample collection after initiation of dosing.

Methods

Animal care and use were conducted in alignment with Animal Welfare regulatory requirements in an AAALAC International accredited facility.

The validation study comprised 3 groups of Wistar Hannover rats with 9 animals/sex for each exteriorisation method. On completion of surgery the TC and VAH animals had a minimum post-operative untethered recovery period of 5 days and the VAB animals 18 days. Each exteriorisation site was flushed twice weekly with 0.5 IU/mL heparin via the pin port.

Following the recovery period, the catheter and tether were passed through the top of the cage and attached to the movable lever arm of the swivel mechanism, used to support the dosing line. Animals were then connected for administration to the infusion pump. Physiological saline was administered at an infusion rate of 4 mL/kg/hr for 91 days.

Bodyweight and food consumption were recorded at least weekly.

TC, VAH and VAB animals were scheduled to be bled from the jugular vein, by needle stick sampling, on days 2, 10, 14, 28 and 56. At terminal necropsy, for bioanalysis, clinical pathology (haematology and clinical chemistry) and/or thyroid hormone analysis. All samples were taken without interruption of infusion.

Animal No./Sex	Route of exteriorisation	Day of termination	Killed or found dead	Reason
R0102M	VAB	Day 64	Killed	Severity of signs
R0103M	VAB	Day 6	Found dead	No signs
R0104M	VAB	Day 32	Killed	Limited use of limbs
R0105M	VAB	Day 67	Killed	Swollen foot
R0109M	VAB	Day 64	Killed	Leaking catheter
R0203M	TC	Day 49	Killed	Snapped catheter unrepairable
R0304M	VAH	Day 69	Killed	Chewed catheter
R0306M	VAH	Day 58	Killed	Chewed catheter
R0308M	VAH	Day 56	Killed	Severity of signs
R0605F	TC	Day 84	Killed	Tailcuff snapped

Table 1. Reasons for early termination.

On completion of treatment, the animals were euthanised by an overdose of pentobarbitone sodium and subjected to a detailed necropsy and histology evaluation of target organs.

Results

Five VAB, 3 VAH and 2 TC animals were removed from study early. The TC group had the lowest mortality rate of 2 animals, both of which were a result of equipment failure (Table 1).

Bodyweight and food consumption were unaffected, with no difference between the three methods of exteriorisation as shown in Figure 4.

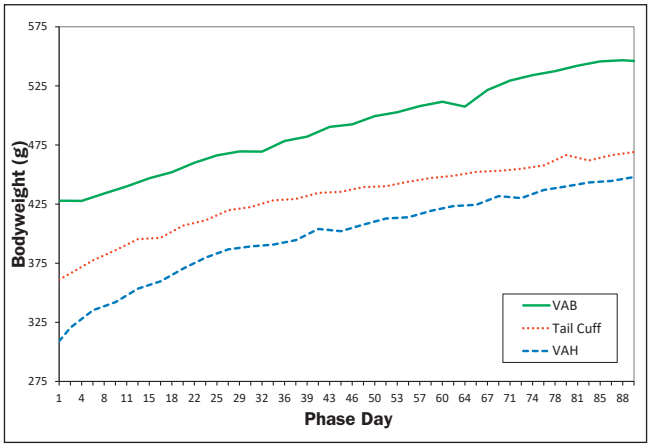

Figure 4. Bodyweight graph.

Blood samples were taken via the jugular route on 19 occasions for all animals in-life, with a maximum of 4 occasions in 24 hours and a maximum volume of 0.8mL. It was not possible to obtain >0.1 mL via jugular for VAH animals on Day 2; therefore, blood samples were changed to tail vein, which required the use of a hot box to vasodilate the tail vessels. For tail vein samples, haematology parameters were affected; red cell counts and associated parameters were reduced. Clinical chemistry samples appeared unaffected by the sampling route.

No significant organ weight changes were observed when compared between groups or historical control range.

Macroscopic changes associated with all techniques were noted but were of least incidence for the TC.

Microscopic examination revealed typically minimal to moderate but occasionally severe, thrombus formation at the catheter tip as a common finding; however for the TC group, only one minor incidence occurred. Minor vascular/perivascular inflammation was noted for TC, VAH animals and VAB females; however this was moderate to severe for VAB males.

		Males			Females		
		2M	3M	4M	2F	3F	4F
Tissue and finding		VAB	TC	VAH	VAB	TC	VAH
Cannula - Tip, Vena Cava	No. examined:	8	8	7	8	7	8
thrombus	Grade -	5	8	3	2	6	5
	+	3	0	4	6	1	3

- = Finding not present; + = present; F = Female; M = Male.
VAB = Vascular Access Button; TC = Tail Cuff; VAH = Vascular Access Harness

Table 2. Incidence of selected findings – 13 week infusion animals.

Conclusion

Following continuous infusion for up to 13 weeks of the three exteriorisation methods, TC had the lowest mortality rate and was least impacted by the infusion technique having the lowest incidence and severity of microscopic findings. Conversely, this laboratory has a long history with the TC model and therefore the surgery and in-life procedures are well established whereas this was the laboratory's first experience with VAB. Therefore although the TC model proved to be the most successful exteriorisation method in this study, the VAB model with further refinement shows great promise.

Required blood sample collection volumes were achieved for TC and VAB but were problematic for VAH. Although volumes were achieved for VAB, some damage was caused to the tissue surrounding the VAB during jugular sampling.

Figure 5. VAH blood sampling attempted via jugular route.

Figure 6. VAB blood sampling achieved via jugular route.

The technique for blood sampling for TC is well established and the difficulties encountered with VAB animals may be confounded by lack of experience but were not impacted by single housing as the animals were of good temperament and easy to handle. The recovery period duration after VAB surgery must be considered to ensure full healing before sampling commences (on day one of dosing).

For all three routes, the moveable lever arm that supported the tether, swivel and line (Figure 7) appeared to decrease pull on the exteriorisation site and resulted in a greater than expected welfare improvement.

Figure 7. Cage setup with lever arm to support dosing equipment.

Acknowledgement

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