Continuous intravenous infusion in mice to support drug development: an update to include juvenile testing

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Introduction

To support development of compounds requiring a constant exposure level, toxicology studies can be conducted with a continuous intravenous infusion method of administration using surgically prepared animals for up to 24 hours a day. Until recently, the rat has been the species of choice for such testing but an alternative species can be the mouse and therefore a mouse vascular infusion model was developed for repeated dosing/long-term toxicity infusion studies. Typically, this would involve adult animals. However based on a requirement to support use of drugs in the paediatric population there is a potential need to dose animals of juvenile age.

This poster will present results showing how vascular continuous infusion in mice with tail-cuff exteriorisation has been developed and refined, along with the successful use of two other infusion models in juvenile aged mice (tail-cuff and vascular access button exteriorisation).

Animal care and use was performed in accordance with Animal Welfare regulatory requirements in an AAALAC International accredited facility.

Tail-cuff exteriorisation refinement

Infusion of freely moving mice can be achieved by surgical implantation of an indwelling femoral vein catheter, which is exteriorised at the tail and protected at the exit point by a steel tail-cuff. The tether-protected catheter is attached to a swivel system outside the animal's cage and connected to an infusion pump. The physical effect of the tail-cuff and the tether must be considered. Securing the tail-cuff by a surgical wire passed through the tail, as well as the weight/drag of the tether and cuff, cause specific tissue lesions.

Continuous infusion in mice via femoral vein catheterisation with tail-cuff exteriorisation has been successfully performed in multiple 14 day toxicology studies at Labcorp in Harrogate. The reliability of this method was not desired, with only an 87% success rate during the dosing period. Permanent tethering (from day of surgical catheter placement) was associated with the early removal of animals due to detached tether and/or tail damage.

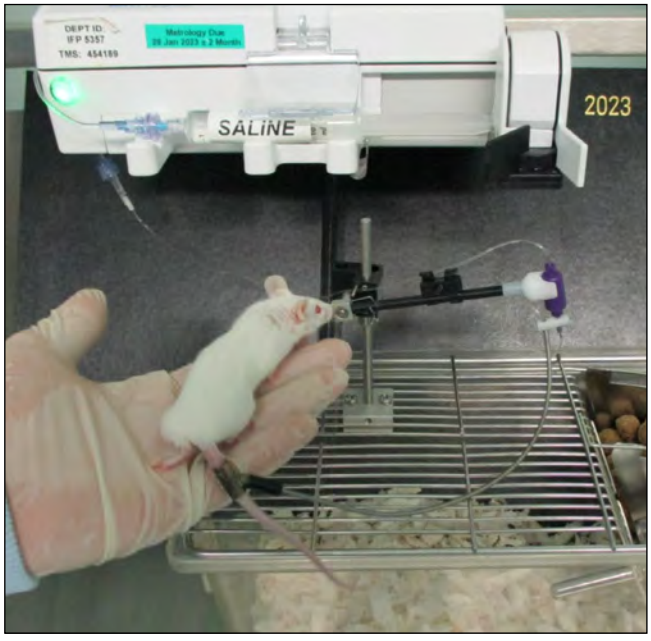

Figure 1. Refinements to continuous infusion in mice.

In this poster, we describe the effect of introducing the following refinements (Figure 1):

- smaller diameter catheter (2 French)
- larger bore surgical wire
- 25-gauge swivel
- flexible moving swivel arm
- reduced handling (daily to twice weekly)

These refinements were implemented in two 14 day continuous infusion studies, total of 374 animals, where the total tethered duration was 19 to 24 days (number of days from surgery to study termination). With the refinements in place, improved bodyweight gain (Figure 2), improved survival rate from 87.6% to 97.9% (Table 1) and decrease macroscopic and microscopic severity of tail lesions were noted.

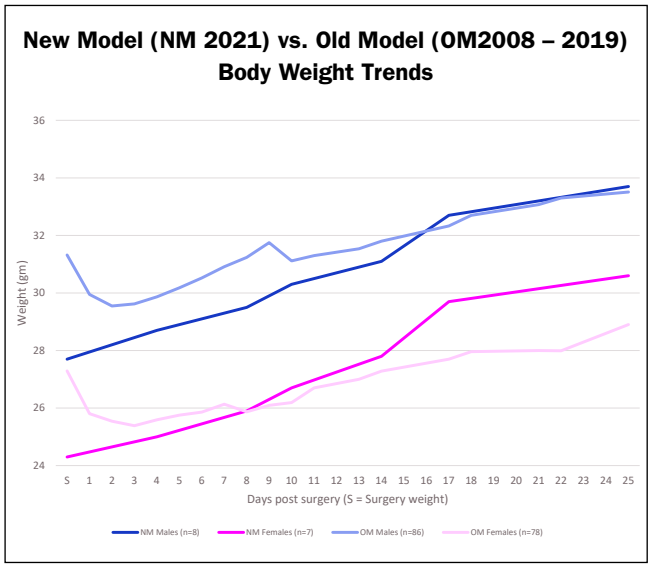

Figure 2. Improved bodyweight gain.

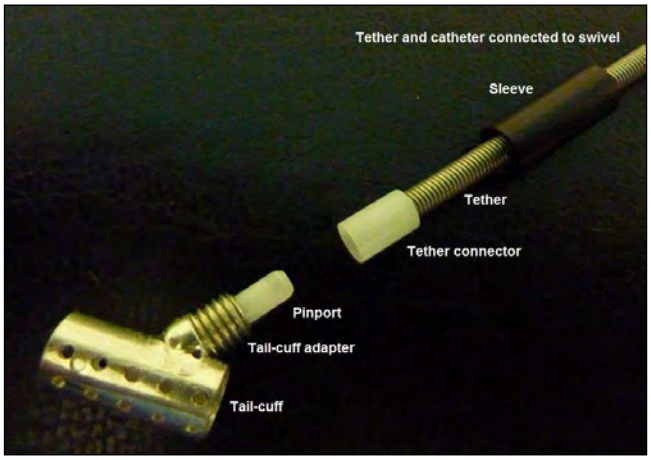

Figure 3. Pinport-in tail-cuff system.

Refinement – utilisation of pinport system

The previously discussed refinements clearly had a positive impact on the infusion delivery system, theorised as largely due to the reduced drag on the tail. To refine this model and to take continuous infusion studies out to 28 days, a 24 animal study was undertaken using the pinport-in tail-cuff system (Figure 3). The benefit of this system was to allow a tether-free surgical recovery period (Figure 4).

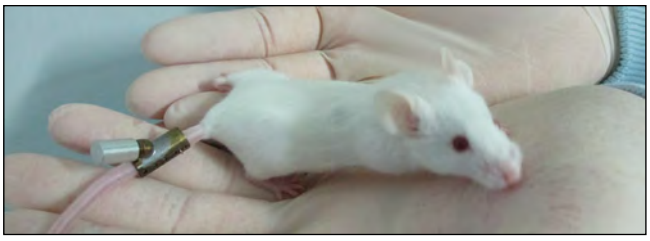

Figure 4. The pinport system allows a tether-free surgical recovery period.

	Old model 14 days		New model 14 days	
	M (169)	F (169)	M (187)	F (187)
Infusion failure	6	13	0	0
Tail damage	4	3	0	0
Exposed cannular	NA	NA	5	2
Detached tether/tail-cuff	3	4	0	0
Bacterial sepsis	NA	NA	1	0
Blocked catheter	2	1	0	0
Poor condition	0	1	0	0
Died during patency check	1	0	0	0
Died after syringe change	0	1	0	0
Leaking catheter	1	2	0	0
Total	17 (10.1%)	25 (14.8%)	6 (3.2%)	2 (1.1%)

Table 1. Improved survival rate using pinport model.

	Method	TC	TC	PP		PP
	Catheter (Fr)	3	2	2		2
	Tethered	Y	Y	N		Y
	Bodyweight	Daily	Daily	Daily	BIW	BIW
	No. females in group	**12**	**12**	**24**	**24**	**12**
Reason for removal	No. examined:	6	4	3*	1*	1*
Catheter detachment/damage	Grade P	2	1	0	0	0
Surgical site swelling	Grade P	0	0	1	0	0
Tail sores/swelling	Grade P	4	3	2	1	1

P = Finding present, BIW = twice weekly, TC = Tail cuff, PP = Pinport-in-tail-cuff study

**Mortality decreased with pinport and with decreased handling*

Table 2. Incidence of reason for removal from 14-day validation.

	Method	TC	TC	PP		PP
	Catheter (Fr)	3	2	2		2
	Tethered	Y	Y	N		Y
	Bodyweight	Daily	Daily	Daily	BIW	BIW
Tissue and finding	**No. females in group**	**12**	**12**	**24**	**24**	**12**
Cannula – port/exteriorisation	No. examined:	10	12	24	24	12
Sore	Grade P	6	6	2*	2*	10
Thick	Grade P	8	9	5*	7*	12
Cannula patency	No. examined:	10	12	24	24	12
Patent	Grade P	3	5	19	13	11
Non patent	Grade P	5	6	5**	11**	1**
Unable to check patency	Grade P	2	1	0	0	0

P = Finding present, BIW = twice weekly, TC = Tail cuff, PP = Pinport-in-tail-cuff study

**Decreased with pinport*

***General trend towards increased patency with 2 French catheter and pinport*

Table 3. Incidence of macroscopic findings from 14 day validation study.

This validation study had a 100% success rate (with all animals reaching the terminal sacrifice), decreased mortality (Table 2, decreased macroscopic findings in the tail (Table 3) and improved patency.

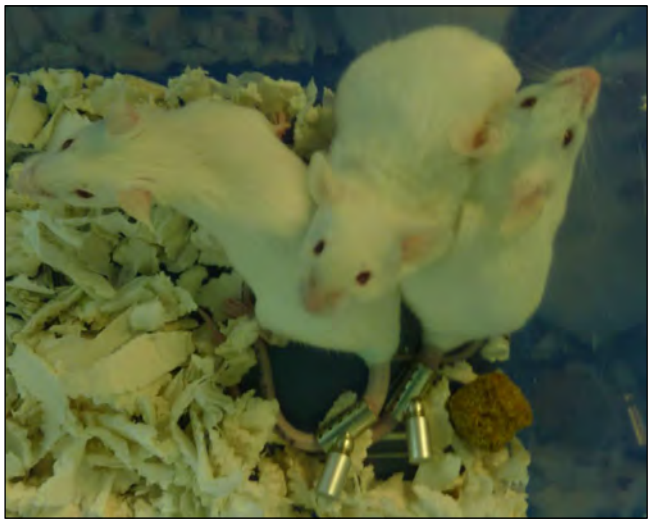

Figure 5. Group housing of animals during surgery recovery.

Figure 6. Use of VAB.

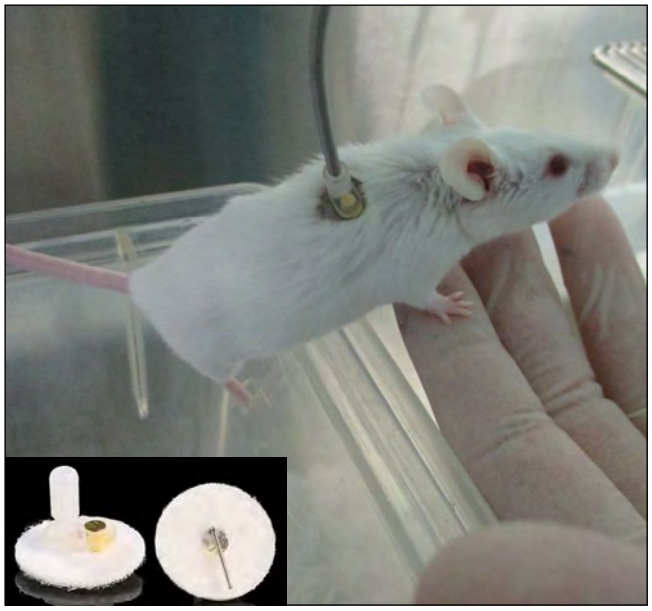

Figure 7. VAB and tether.

Discussion and conclusion

This poster demonstrates the refinements for the tail-cuff continuous infusion system, and the significant advancements achieved at Labcorp in Harrogate. The methodology offers the option to disconnect the tether during the recovery period after surgery, allowing reduction of total tether period on continuous infusion studies. There was a reduction in lesions and 100% survival achieved on the 28 day study, compared to 74% survival with the permanent tethered non-refined infusion system.

Alongside refinements to the tail-cuff exteriorisation, group housing of animals during surgery recovery (Figure 5) and use of the vascular access button (VAB, Figures 6 and 7) is being investigated. Good results have been achieved so far with the VAB model; further refinement and experience development are required.

Development of a mouse continuous intravenous infusion model in juvenile animals

Some drugs that are developed for use in children may require juvenile animal testing. If there is a need for use for continuous infusion and the tox species is the mouse, these models would be selected for validation.

Mice can be weaned on day 14 when given twice daily wet pellets, and although this leads to a reduced bodyweight, Bailoo *et al*. demonstrated that this effect was only apparent up to the age of 26 days, at which time animals weaned at day 14 were of a similar weight to animals weaned at 18, 22 or 26 days of age (Scientific reports 10, article number 11684, published 15 July 2020, Jeremy D. Bailoo, Bernard Voelkl, [https://www.nature.com/articles/s41598-020- 68549-3]).

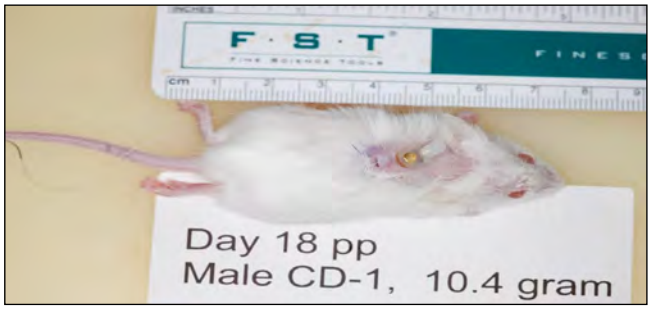

Figure 8. Juvenile mouse with VAB (18 days of age).

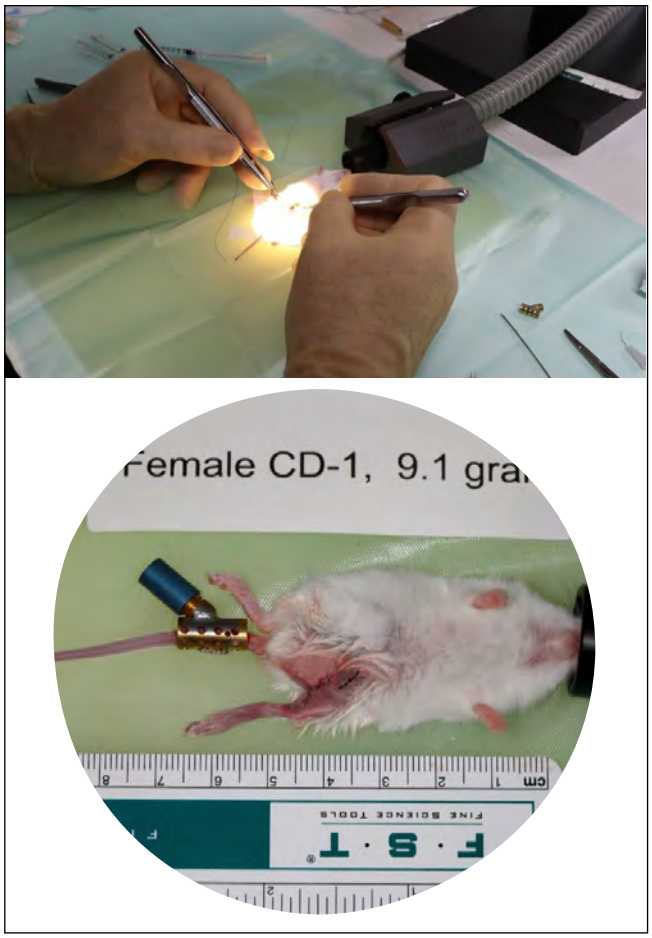

Figure 9. Femoral vein juvenile mice 17 days of age cannulated, micro forceps 100 micron.

On this basis 8 juvenile mice (17 days old and between 9.1g to 10.4g) were successfully catheterised and most survived an observation period of 24 hours before scheduled euthanasia. The mice recovered, group-housed, without the mother and gained approximately 1g of weight over the observation period. The mice catheterisation was not performed at Labcorp but competency of the surgeon was assured. Animals can therefore be prepared for drug infusion (after recovery) at 3 weeks old which is often considered in rodents to be equivalent to a 2 year-old human child.