Surgery Refinements Improve Success Rates in Rat Bile Collection

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Introduction

Rats are typically the rodent species of choice for investigating the absorption, distribution, metabolism and excretion of new chemical entities. In the absence of a gall bladder, they are ideal for researching the time-course of excretion and metabolism of novel xenobiotics in bile.

This poster compares the model success rates using 3 different housing regimes during recovery from surgery.

The experimental study phase of all 3 methods remained unchanged – animals were tethered and singly housed in glass metabolism cages in order to separately collect bile and excreta and obtain an excretion balance for the duration of the study (up to 96 hours).

Only animals deemed to be healthy and with a good bile flow were used on study. Bodyweights and clinical signs, including assessment of urine, faeces and bile output were recorded during the recovery period to monitor the health of the animals for animal welfare.

Methods

Bile and Duodenum Cannulation Success Rates		
Year		**Model**
1997-2000	Method 1	Dual Cannulation - Direct Catheters
2011-2016	Method 2	Dual Cannulation - Direct Catheters
2016-2019	Method 3	Dual Cannulation - PinPort™

Rat strains used in this poster were Harlan Hanover Wistar, Charles River Sprague Dawley and Hanover Wistar.

The same surgical technique was used for all 3 methods. Catheters were placed in the bile duct to collect bile; a second catheter was secured into the duodenum where artificial bile salts were infused. Both catheters were exteriorised via a tail cuff.[1]

Method 1 – animals were tethered and singly housed in glass metabowls from surgery until the end of study (not performed at this establishment). [2]

Method 2 – Animals were allowed to recover from surgery, singly housed in a standard cage whilst being tethered. They were transferred to a glass metabowl the day before dosing.[3]

Method 3 – A modified tail cuff was developed which enabled both catheters to be passed through the tail cuff and connected to the dual PinPort™. The U-shaped loop was connected to the dual port permitting the animal to recirculate its own bile back into the duodenum during the recovery period. The port was protected by a cap screwed onto the tail cuff. This modification meant that animals did not need to be tethered and could be group housed with standard environmental enrichment in standard caging. It also enabled the recovery period to be extended and therefore to allow the animals more time to recuperate from the surgical procedure

Following surgery, the animals were returned to group housing and their health and welfare monitored during the recovery period (Figure 1)[4].

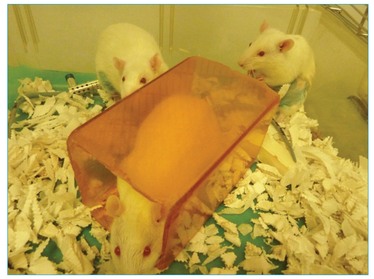

Figure 1. Group-housed animals that are untethered with the PinPort™ cannula transferring the animal's own bile between the bile duct and duodenum.

When bile was to be collected for analysis, the U-shaped loop was removed from the port within the tail cuff adapter (Figure 2). and a dual tether fixed onto the dual port. The exteriorised catheters from the tether were connected to a dual stainless steel swivel device. Animals were then singly housed in metabolism cages to enable bile, urine and faeces to be collected for periods of up to 96 hours (Figure 3). Artificial bile salts were infused through the duodenal cannula to replace the bile collected, animals were continuously tethered for the duration of the study.

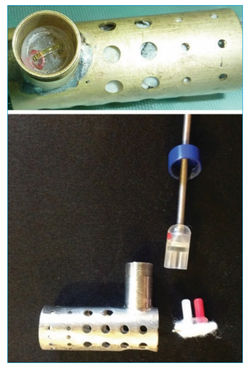

Figure 2. The PinPort™ tail cuff in-situ during the recirculation phase.

Figure 3. Tethered rat in a metabolism cage during the sample collection period.

Results

The surgery success rate for method 1 was not recorded, surgery success rates for methods 2 and 3 were >99%.

Table 1 shows the percentage of animals suitable for starting the study and the percentage of animals completing the study.

Tethering/housing conditions and associated success rates are shown in Tables 2 and 3.

Comparison of procedural issues between method 2 and method 3: Table 4.

Table 1. Bile Duct (Bile and Duodenum) Cannulation Success Rates

Bile Duct (Bile and Duodenum) Cannulation Success Rates					
Year		**Model**	**No. of Animals Prepared**	**% Suitable for Study**	**% Achieving End of Study**
1997-2000	Method 1	Dual Cannulation - Direct Catheters	266	74	Not Recorded
2011-2016	Method 2	Dual Cannulation - Direct Catheters	392	87	82
2016-2019	Method 3	Dual Cannulation - PinPort™	288	90	77

Table 2. Housing Conditions for Different Models

	Single Housed in Glass Metabowls No of Days	Continuously Tethered in Standard Cage No of Days	Group Housed with Env. Enrichment No of Days	Study - Single Housed in Glass Metabowls No of Days
Method 1	5	0	0	3
Method 2	0	3	0	3
Method 3	0	0	6	4

Table 3. Success Rates in Tethering/Housing Condition Post-Surgery

	No. Animals Surgically Prepared		
Method 1	266	Continuously Tethered, Single Housed in Glass Metabowls	
		5 Day Recovery (74% Success Rate)	Not Recorded
Method 2	392 (3)	Continuously Tethered in Home Cage (No Environmental Enrichment)	Single Housed in Glass Metabowls
		3 Day Recovery (87% Success Rate)	Average 72 Hour Study (94% Success Rate)
Method 3	288 (1)	Standard Caging, Group Housing, Standard Environmental Enrichment	Single Housed in Glass Metabowls
		6 Days Recovery (90% Success Rate)	Average 96 Hour Study (85% Success Rate)

The dropout rate during the surgery/recovery period for method 2 was 13%, against 10.1% for method 3. The recovery period has been extended from 3 days (method 2) to 6 days (method 3).

Extending the length of the recovery/study period to 10 days for method 3 increases amount of time for problems to occur.

The overall dropout during surgery, recovery and study periods for method 2 (over 6 days) is 12% per day, compared to method 3 (over 10 days) 6.7% per day.

Table 4. Surgery and Study-Associated Issues - Number of Animals (%)

	2011-2016 - Direct Catheters		2016-2019 - PinPort ™	
	During Recovery	**During Study**	**During Recovery**	**During Study**
Died during surgery	3 (0.8)		1 (0.3)	
Incorrect port connection				1 (0.4)
Snapped catheter	4 (1.0)	1 (0.3)	3 (1.0)	3 (1.2)
Bile leaking from tail cuff (inc. catheter detached from port)			6 (2.1)	3 (1.2)
Tether/catheter chewed	16 (4.1)	7 (2.1)	1 (1.0)	1 (0.4)
No bile flow	10 (2.6)	0		20 (7.7)
Poor condition/weight loss or both	16 (4.1)	10 (2.9)	16 (5.6)	8 (3.1)
Found dead	1 (0.3)	1 (0.3)	2 (0.7)	
Unknown	1 (0.3)			
Mis-dosed		2 (0.6)		2 (0.8)
Total	51 (13.0)	21 (6.2)	29 (10.1)	38 (14.8)

The incidence of poor condition/bodyweight loss after surgery and the number of snapped catheters were similar for methods 2 and 3.

The number of losses attributed to the permanent tethering of animals in method 2 were higher due to the difficulty of performing repairs to chewed tethers although animal losses due to no bile flow was lower in method 2 compared to method 3. Note that in method 2 no connectors were used. All losses were euthanised in accordance with humane end points.

During the development of method 3, a number of animals were lost due to the catheter becoming detached from the port during tethering, this reduced over time as experience with the method was gained. It has been observed that bile from animals was still flowing when the connector was removed from catheter at necropsy.

However it is clear that the welfare improvements in method 3 are enormous as evidenced by the improved body weight gain in PinPort™ animals (method 3) when compared to the other methods[4].

The surgical success (measured by the number of animals producing acceptable bile flow after a recovery period) was comparable to the continuously tethered model (method 2).

Conclusions

Surgical success rate, reduction in bodyweight losses, comparable animal health observation, and acceptable bile flow show that the PinPort™ model hugely improves animal health and welfare without infringing scientific integrity[4].

References

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