Continuous Infusion Toxicity Studies in Rats: Experiences and Developments at Harlan Laboratories Switzerland

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26 February 2009
Continuous Infusion Toxicity Studies at Harlan Laboratories

1. Why do you need infusion studies?
2. History of infusion studies at Harlan Laboratories
3. Development of the cage system
4. Selection of the pump
5. Externalization of the catheter
6. Fine tuning - Validation studies
7. Outlook
Why infusion studies?

Increasing need for infusion studies over the last 5 to 7 years

- New drugs are frequently proteins
- Cancer treatment (intermittent dosing in treatment cycles)
- Acute treatment with e.g. antibiotics
Cage System: Tail Cuff Method

Makrolon type III cage

Catheter is fixed with a metal coil on the base of the tail

Catheter in femoral vein (surgery done by Dr. Klaus Weber at Harlan/RCC)

Used at Harlan/RCC until 2002
Cage System: Tail Cuff Method

Problems:
- Bedding material accumulates between metal coil and skin of the tail
- Animals manipulate the metal coil/ try to get rid of it
- Tails are swollen due to mechanical abrasion

Consequences:
→ Local irritations and open wounds on the tail
→ Inflammations at the catheter entry site, tail necrosis
→ Technique-related loss of animals before the end of the study
→ Need to start with 50% more animals than needed for evaluation to compensate for losses
Cage System: Dilab AccuSampler Cage

Main difference to the old cage system:

Catheter is **exteriorized in the subscapular** region of the animal through an implantable skin button (Dacron button)

The animals are **tethered with a spring coil** to a swivel which allows the animal uninhibited movement => better acceptance by the animals

Initial work with this cage highlighted some problems  (e.g. “cage tourism”)
Cage System: Modified Dilab AccuSampler Cage
Cage System: Modified Dilab AccuSampler Cage

Modifications:

1. One half of the lower cage part painted in black
2. A ventilated plastic cage top with an arch cut to allow the tether to move freely
3. Fixation buttons
4. Ventilation holes
Infusion Pump

Braun Perfusor Compact S®, Braun GmbH, Germany

- Guarantees definitive start and end points for treatment and recovery periods, an important consideration for GLP studies

- Pump has a back pressure sensor that acoustically indicates possible clotting problems
An animal room for Infusion Tox Studies at Harlan Laboratories
Validation Study No. 1 “4-week infusion”

Strain: Wistar

Catheterization: Polyurethane catheter in the *Vena jugularis*

Catheter attachment: Dacron button

Breeding & operation: By an external supplier

Treatment period: 28 days

Infusion: 24 hours

<table>
<thead>
<tr>
<th>Group</th>
<th>Substance</th>
<th>Dose Volume (ml/kg/h)</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5% glucose</td>
<td>1</td>
<td>5♂/5♀</td>
</tr>
<tr>
<td>Group 2</td>
<td>5% glucose</td>
<td>2</td>
<td>5♂/5♀</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.9% saline</td>
<td>1</td>
<td>5♂/5♀</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.9% saline</td>
<td>2</td>
<td>5♂/5♀</td>
</tr>
</tbody>
</table>
Validation Study No. 1 “4-week infusion”: Results

The study had to be terminated early because of a high incidence of premature mortalities due to

a) Poor hygienic conditions during surgery

b) Incorrect catheterization – in several animals the catheter tip reached the atrium

The supplier was invited for a discussion of these problems and the surgery procedures were adopted according to the RCC SOP for catheterization

→ A new validation study was initiated
Validation Study No. 2 – “4-week infusion”

Strain: Sprague Dawley (*considered to be superior to Wistars due to the higher BW at surgery*)

Catheterization: Polyurethane catheter in the Vena femoralis

Catheter attachment: Dacron button

Breeding & operation: By an external supplier

Treatment period: 29 days

Infusion: 24 hours

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<tr>
<td>Group 1</td>
<td>0.9% saline</td>
<td>1</td>
<td>6♂/6♀</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.9% saline</td>
<td>2</td>
<td>6♂/6♀</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.9% saline</td>
<td>3</td>
<td>6♂/6♀</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.9% saline</td>
<td>4</td>
<td>6♂/6♀</td>
</tr>
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</table>
Validation Study No. 2 – “4-week infusion”

Monitoring:

During the treatment period:

Viability/ mortality/ clinical signs  twice daily

Food consumption/ body weights  twice weekly

At the end of the treatment period:

Blood sampling for clinical laboratory investigations

Urine sampling
Validation Study No. 2 – “4-week infusion”

Modification of metabolism cages allowed continuous infusion during the urine collection period

One half of the cage painted in black
Validation Study No. 2 – “4-week infusion”

Full necropsy of all animals

Macroscopic abnormalities were recorded and various organ weights were determined (according to international guideline)

A full set of tissues was collected and slides were prepared for histopathological examination (according to international guideline)
Validation Study No. 2 – “4-week infusion”: Results

No animals died during the study.

Food consumption and body weight were not affected by the infusion method or the infusion rate.
Validation Study No. 2 – “4-week infusion”: Results

Measurement of food consumption

Powder feed was used in a hanging feed box. The box was knocked down by six animals.

For subsequent studies a metal feed rack containing pelleted feed screwed tightly to the cage wall were used.
Validation Study No. 2 – “4-week infusion”: Results

Clinical Laboratory Investigations

→ Most of the measured parameters were within our reference ranges (historical control data)

→ Some values were, unsurprisingly, on or slightly outside our reference ranges from non-catheterized control Sprague Dawley rats.

But: No differences between the groups
Validation Study No. 2 – “4-week infusion”: Results

Pathology

Microscopically, a few animals from all groups showed pathogenesis around the point where the catheter entered the blood vessel:

Phlebitis, periphlebitis and thrombophlebitis.

→ well known and inevitable result of inserting a catheter into a blood vessel and not considered to be an adverse effect
Validation Study No. 2 – “4-week infusion”: Results

**Blocking of the catheter**

→ Occurred 25 times throughout the study

→ 19 clots could be removed successfully into a syringe filled with physiological saline

→ 6 catheters were treated with human urokinase (2000 IU in 400 μl over 20 min)

The use of urokinase to free clots formed at the catheter tip was considered to be a suitable method with no apparent effect on clinical pathology parameters.

**But:** urokinase treatment can only be performed once per animal (due to possible immune reactions)
Validation Study No. 2 – “4-week infusion”: Results

Catheter attachment
Towards the end of the study the Dacron button detached in 11 animals.

→ The Dacron button was replaced by a harness in these animals

→ → For studies longer than 14 days a harness is preferred.
Validation Study No. 2 – “4-week infusion”: Results

Infusion rates

Infusion rates of 0.9% NaCl at 1, 2 or 3 ml/kg/hour considered suitable (no relevant microscopic changes at the infusion site).

An infusion rate of 0.9% NaCl at 4 ml/kg/hour induced the highest gradings of periphlebitis and thrombophlebitis in single rats at the catheterization site and also some inflammatory processes in organs other than the catheterization site.

→ This infusion rate was not considered to be suitable for such studies.
Validation Study No. 2 – “4-week infusion”: Conclusions

1.) Infusion studies with Dacron button attachment can be performed for up to 14 days of treatment

2.) A dose volume of up to 3 ml/kg/h is recommended

3.) Human urokinase can be used to clear clotted catheters

4.) Sprague Dawley rats are the better model (… but just for infusion studies)
Validation Study No. 3 “13-week infusion”

Strain: Sprague Dawley
Catheterization: Catheter in the Vena jugularis
Catheter attachment: Instech Solomon Infusion Harness (Groups 1-3)
Instech Solomon Vascular Access Harness (Group 4)
Breeding & operation: Harlan Laboratories, B.V., The Netherlands
Treatment period: 13 weeks
Infusion: 24 hours (4 hours)

<table>
<thead>
<tr>
<th>Group</th>
<th>Substance</th>
<th>Dose Volume (ml/kg/h)</th>
<th>No. of Animals</th>
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<th>Infusion</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>0.9% saline</td>
<td>3</td>
<td>10♂</td>
<td>polyurethane</td>
<td>24 hours</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.9% saline</td>
<td>3</td>
<td>10♂</td>
<td>silicon</td>
<td>24 hours</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.9% saline</td>
<td>3</td>
<td>10♂</td>
<td>silicon round tip</td>
<td>24 hours</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.9% saline</td>
<td>3</td>
<td>10♂</td>
<td>silicon round tip</td>
<td>4 hours/day</td>
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Validation Study No. 3 “13-week infusion”

Groups 1 - 3

Instech Solomon Infusion Harness:

Tube to the pump is permanently connected to the harness
Validation Study No. 3 “13-week infusion”

Group 4

Instech Solomon Vascular Access Harness (VAH):

Plug / socket principle
Validation Study No. 3 “13-week infusion”: Results

General observation: animals with harnesses are more active than animals with Dacron button fixation during the first two weeks (Dacron button is fixed on the neck muscles).

Rapid body shaking led to forces impacting on the whole catheter system: catheter damage
Validation Study No. 3 “13-week infusion”: Enrichment

Paper strips enabling nest building and hiding from light during the light phase were used to calm the animals.
Validation Study No. 3 “13-week infusion”: Results

- **Group 2: Silicon**
- **Group 3: Silicon round tip**
Validation Study No. 3 “13-week infusion”: Results

Groups 2 and 3 (animals with silicon catheters):

→ No animal reached the end of the treatment period

Reason for the termination of animals

1.) Swellings near catheter entry site – 8/20 animals*
2.) Catheter ripped out of vein – 5/20 animals
3.) Spontaneous death – 3/20 animals*
4.) Catheter detachment – 2/20 animals (see next slides)
5.) General bad condition – 2/20 animals*

* (Reasons to be evaluated based on histopathology data)
Validation Study No. 3 “13-week infusion”: Detachment problem

Thick-walled polyurethane tube coming from the infusion pump

Metal coupler

Thin-walled silicone tube running into the jugular vein
Validation Study No. 3 “13-week infusion”: Detachment problem

Hydrostatic pressure
In only 2/20 animals “catheter detachment” was the proximate cause of terminating the animal. However “catheter detachment” occurred in a total of 15/20 animals – in some animals more than once.

→ A surgical operation was necessary - animals had to be anaesthetized and the harness detached. The surgeon had to “search” for the catheter end between muscle and skin of the animal and reconnect it to the metal coupler.

→→ additional stress for the animal

→→→ the “catheter detachment” maybe directly or indirectly related to other causes which made the termination of animals necessary
Validation Study No. 3 “13-week infusion”: Results

- **Group 1:** Polyurethane
- **Group 2:** Silicon
- **Group 3:** Silicon round tip

![Graph showing the number of animals over treatment days for three different groups.](image-url)
Validation Study No. 3 “13-week infusion”: Results

Group 1 (animals with polyurethane catheters):

→ No animal reached the end of the treatment period

Reason for the termination of animals

1.) Swellings near catheter entry site – 4/10 animals
2.) Catheter ripped out of vein – 4/10 animals
3.) General bad condition – 1/10 animals
4.) Catheter detachment – 1/10 animals
Validation Study No. 3 “13-week infusion”: Results
Validation Study No. 3 “13-week infusion”: Results

Group 4 (animals with Vascular Access Harness):

7/10 reached the end of the treatment period (90 days)

Termination of animals
1st animal – day 66
2nd animal – day 84
3rd animal – day 85

Reason for the termination of animals
1.) Catheter ripped out of vein – 2/10 animals
2.) Defective catheter – 1/10 animals
Validation Study No. 3 “13-week infusion”: Conclusions I

→ Cause of the catheter detachment was identified too late, no animals in groups 1, 2 or 3 reached the planned necropsy date

→ Vascular Access Harness (VAH) proved to be best solution: Animals can be disconnected for recording of body weights and blood sampling, VAH seems to be more resistant to sudden movements of animals

→ No occlusions of the catheters occurred during the disconnected periods in animals with VAH (catheters were not flushed during the disconnected time)

However: animals in groups 1 – 3 were infused over 24 hours, animals in group 4 (VAH) only 4 hours /day
Validation Study No. 3 “13-week infusion”

Strain: Sprague Dawley
Catheterization: Catheter in the Vena jugularis
Catheter attachment: Instech Solomon Infusion Harness (Groups 1-3)
Instech Solomon Vascular Access Harness (Groups 4-6)
Breeding & operation: Harlan Laboratories, B.V., The Netherlands
Treatment period: 13 weeks
Infusion: 24 hours (groups 1, 2, 3, 5, 6), 4 hours (group 4)

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<td>3</td>
<td>10♂</td>
<td>silicon round tip</td>
<td>4 hours/day</td>
</tr>
<tr>
<td>Group 5</td>
<td>0.9% saline</td>
<td>3</td>
<td>5♂/5♀</td>
<td>silicon round tip</td>
<td>24 hours</td>
</tr>
<tr>
<td>Group 6</td>
<td>0.9% saline</td>
<td>3</td>
<td>5♂/5♀</td>
<td>polyurethane</td>
<td>24 hours</td>
</tr>
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Validation Study No. 3 “13-week infusion”: Results

- **Group 5: VAH Silicon round tip**
- **Group 6: VAH Polyurethane**

The graph shows the number of animals remaining in each group over the treatment days.
Validation Study No. 3 “13-week infusion”: Results

Reason for the termination of animals (group 5)
1.) Catheter ripped out of vein – 2/10 animals
2.) Defective catheter – 2/10 animals
3.) Swellings – 3/10 animals

Reason for the termination of animals (group 6)
Catheter ripped out of vein – 1/10 animals
Spontaneous death – 1/10 animals*
General bad condition (Body weight loss, ruffled fur, etc.) – 1/10 animals

* (Reason to be evaluated based on histopathology data)
Polyurethane catheter seems to be superior to the silicon catheter

4-week infusion studies can/should be performed with VAH without loss of animals related to the study design
**Overall Conclusions**

After 7 years development of infusion studies we recommend:

1. Modified Dilab AccuSampler Cage with half of the lower part painted with a dark color, ventilation holes, removable plastic top, and a food rack with food pellets

2. Infusion pumps with back pressure sensor (superior to osmotic pumps)

3. Catheterized animals from Harlan Laboratories, B.V., The Netherlands (preferentially Sprague Dawley rats)

4. Jugular vein as catheter entry site

5. For studies with treatment up to 14 days: Dacron button (cutting the catheters for recovery period)

6. VAH with polyurethane catheters for studies longer than 14 days
Outlook

Use of Vascular Access Harness is the most suitable externalization technique for infusion studies

Use of Fresenius Kabi infusion filter with air separator to avoid accidental injections of air bubbles
Outlook

Can studies of 13 weeks be performed with VAH and a polyurethane catheter?

Why is polyurethane better than silicon?

What can be done to minimize the animal losses towards the end of the treatment period? Why did so many animals rip out their catheters towards the end of the treatment period?

Evaluation of clinical diagnostic and histopathology data of the 13-week validation study are currently underway.
Thank you!

Dilab (Sweden)

Instech Solomon (USA)

Harlan Laboratories Ltd.
Roland Sacher, Alexander Kohler, Andreas Otto, Ian Bunn