Common Rodent Techniques used in Cancer Models

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Laboratory Animal Sciences Program, June 2018
Overview

- Identification, Handling and Restraint of mice
- Drug administration and injections
- Blood collection
- Anesthesia and Analgesia
- Final Comments
Male vs Female

Anogenital Distance is longer in Males vs Females
Restraint

Manual Restraint

One-handed Restraint Method

Two-handed Restraint Method

Restraint Device
Take advantage of the mouse’s normal tendency to pull away from you

Always Grab near base of tail
A properly restrained mouse

- Successful procedures
- Do not attempt injection or gavage if mouse is struggling
Restraint

- Monitor breathing rate and membrane color (cyanosis)
- Do not throw, spin, or threaten mice
- Light restraint is sometimes best
  - Strains dependent
- If a mouse is dropped on floor or escapes, alert facility supervisor
Identification of Mice

- Critical for genotyping
- Indelible, non-toxic markers (Sharpie) may last 3-4 days; lines or dots on tail

Cage Card

Indelible Marker
Identification – Ear punch/notch

- Ear punch or ear notch (also use for genotyping)
- 10-15 days of age: ear punch.

*Wendy Dubois*
Identification - Ear Tags

- Ear tags should be sanitized and cleaned with alcohol.
- After 18 days of age: ear tag
Identification - Tattoo

- Tattoo: any age ($$ equipment)
  - Tattoo toes, rather than toe-clip
Identification - Microchip

- Bio Medic Data Systems, Inc.,
- AVID

BMDS Exhibit at SOT 2018
BMDS is proud to announce we will be exhibiting at the 57th SOT Annual Meeting. Booth, Number 631

Featured Products
- DAS-8001
  Our New Premier Data Collection System. Desktop or Portable, your choice.
- DAS-8006
  Wireless Reader System for IMI Micro Transponders
Tissue collection for genotyping

- Select the genotyping method appropriate for your research purposes.
- Tail snip between 10-21 days old
  - 2-5 mm tail excised
  - Ethyl chloride spray or ice cold ethanol
  - Begin with sterile instruments and sanitize between animals
  - Older mice (>21 days): must use anesthesia
Figure 1. Schematic diagram illustrating the anatomy of the rat respiratory and gastro-esophageal tracts. During gavage dosing the gavage needle delivers the dosing formulation near the gastro-esophageal sphincter. In cases where there is a high volume content in the stomach (e.g. due to a full stomach) there may be reflux of the administered test formulation and food back up the esophagus upon removal of the gavage tube. During aspiration test formulation can then enter from the laryngo-pharynx into the nose (retrograde aspiration) particularly in rats as they are nasal breathers and are not able to breathe through their mouth.
Drug delivery – Oral Gavage

- Safe guideline is 1% body weight (e.g. 20 g mouse receives 0.2 ml)
Drug Delivery – Oral Gavage

• Oral gavage:
  • Technical expertise required to prevent injury – esophageal rupture
  • Should be very smooth with no resistance

Turner et al, JAALAS, 2011
Subcutaneous Injection

- Tumor implantation
  - Flank or mammary fat pad
  - +/- Matrigel
  - Tumor fragments (~3 mm³)

- Drug Delivery

- Supportive Care
  - Following surgery or whole body irradiation
  - Fluid therapy, analgesics, antibiotics etc.
  - Use only isotonic NaCl or LRS for fluid therapy
  - Prewarm fluids to prevent hypothermia
Subcutaneous injection

- Must be nonirritating
- Rapid, inexpensive
- May be absorbed slower compared to other routes
- Restrain, make a dorsal tent of skin over scruff
- Insert needle bevel-up in retracted loose skin over neck and back and aspirate to assure proper placement
- Recommend volume ~0.25 ml
- Max volume 1.0 ml
  - Depending on site

Turner et al, JAALAS, 2011
Intradermal injections

- Mouse should be anesthetized
- Remove hair by shaving or plucking & swab with 70% alcohol
- Insert 27-30G needle into skin, bevel-up, nearly parallel to skin. Do not aspirate.
- Inject no more than 50 μl
- Bleb should be evident

DVR SOP 216
Intramuscular injection

- Recommended volume 0.05 ml
- Quadriceps is best
- Avoid sciatic nerve
- Orthotopic muscle tumor cell injections
- Absorption rate similar to SC
Intraperitoneal injection

- Rapid absorption may closely resemble iv injection kinetics for some compounds
- Use the lower left or right quadrant
- Tilt the head down
- Insert needle at 30° angle
- Recommend ~0.5ml
- Slight negative pressure ensures correct placement
Intravenous injection – Tail Vein
Intravenous injection – Retroorbital

Lab Animal 40, 155–160 (2011)
Intracardiac Injections

- Technically challenging – get some help & know your anatomy and SOP
- Delivery metastases systemically e.g. brain or bone
- Under anesthesia → left ventricle
- Single cell suspensions
- ~0.1 ml ~100,000 cells
- Nudes much easier than haired mice
- Acute mortality may be seen
- Evaluate for accuracy
  - imaging/post mortem
Intracardiac injection – Ultrasound-guided
Topical drug application

- Skin should be shaved/clipped (maximize contact) and cleaned but not traumatized
- Application with pipette, applicator stick
- Difficult preventing ingestion of compound – caution systemic toxicity
- Cover with bandage and consider single housing
- Consider soft, dust-free bedding
- Consider: skin intact, skin thickness, skin hydration
Injection Needle Sizes

- Reference: LASP website & ACUC guidelines
- Factor in viscosity and site
- Length: ½ to 1 inch
- IV (tail vein) <25 ga
- SC < 23 ga
- IM < 25 ga (27)
- IP < 25 ga (25)
- ID < 27 ga
- Risk of aerosolization
  - Luer-lock; Luer-slips
  - Eye protection

http://www.fda.gov/MedicalDevices/Safety
Repeat injections with same needle?

• Needles through skin/stopper dull the tip
  ● More painful & less effective
  ● Contaminated needle can transmit disease

• We recommend changing needles between every animal
  ● This may not always be practical

• No more than 5 mice injected per needle
  ● Depending on needle size, substance administered etc
  ● Mice should be from same cage
Alternatives to multiple injections

How does it work?

ALZET pumps operate because of an osmotic pressure difference between a compartment within the pump, called the salt sleeve, and the tissue environment in which the pump is implanted. The high osmolality of the salt sleeve causes water to flux into the pump through a semipermeable membrane which forms the outer surface of the pump. As the water enters the salt sleeve, it compresses the flexible reservoir, displacing the test solution from the pump at a controlled, predetermined rate. Because the compressed reservoir cannot be refilled, the pumps are designed for single-use only.

The rate of delivery by an ALZET pump is controlled by the water permeability of the pump's outer membrane. Thus, the delivery profile of the pump is independent of the drug formulation dispersed. Drugs of various molecular configurations, including ionized drugs and macromolecules, can be dispensed continuously in a variety of compatible vehicles at controlled rates. The molecular weight of a compound, or its physical and chemical properties, has no bearing on its rate of delivery by ALZET pumps.

The volume delivery rate of ALZET pumps is fixed at manufacture. ALZET osmotic pumps are available with a variety of delivery rates between 0.11 and 10 µL/hr and delivery durations between 1 day and 6 weeks. While the volume delivery rate of the pump is fixed, different dosing rates can be achieved by varying the concentration of agent in the solution or suspension used to fill the pump reservoir.

A more complete and technical explanation of the operation of ALZET osmotic pumps can be found in the following reference.


Learn More About...
- ALZET Catheters
- Ordering Online
- Rates & Durations
- Technical Specifications

Injection vs. Infusion

OVERDOSAGE
- Side effects
- Drug waste

THERAPEUTIC DOSE

UNDERDOSE
- Loss of effect

TIME, IN HOURS

0 24 48 72 96

DRUG LEVEL

ALZET DOSING
CONVENTIONAL DOSING

Download publication
Alternatives to multiple injections

• Slow-release pellets
  ● E.g. Innovative Research of America


- Ultrasound Imaging-guided Intracardiac Injection to Develop a Mouse Model of Breast Cancer Brain Metastases Followed by Longitudinal MRI. *J Vis Exp.* 2014; (85): 51146.
Blood collection
Complete blood count and chemistry

- Yes it is done in mice
- LASP Pathology/Histotechnology Laboratory
  - Communicate with lab before collecting samples
    - 2 days notice,
  - EDTA microvette (e.g. 100ul, sarstedt, 20.1278.100)
  - Clinical chemistry - Abaxis VetScan, Vitros 250
- LASP Bethesda has:
  - iSTAT and Abaxis Vetscan
- Clinical chemistry and hematology analytes
  - 100-200 ul will likely need to be collected for each
Blood collection

• How much blood can I take from a mouse?

• Answer: it depends…
  ● check your protocol,
  ● the size of the mouse,
  ● the frequency and interval of collections
  ● whether or not replacement fluids are given
  ● whether it is a terminal blood collection
Blood Collection

- Large volumes (e.g. 300-500ul)
  - Done under anesthesia
  - Generally terminal e.g. cardiac bleeds

- Smaller volumes (e.g. ~100ul)
  - OACU Guidelines for Survival Bleeding of Mice and Rats
  - ~10% TBV per 2-4 wks
  - 110-140 ul for a 20g mouse
  - >10% q 2wks → fluid replacement & ACUC justify
Dorsal Pedal vein
Performed Awake, good for repeat smaller vols.

Tail Nick
Performed Awake, small vols.

Cardiac Puncture (non survival)
Anesthesia required, know anatomy
0.5-1.5ml

Retro-orbital Sinus
Anesthesia required

Mandibular vein
Performed Awake, reliable
Best for large volumes
100-300ul

Jugular vein
Anesthesia required

Saphenous veins
medial & lateral
Performed Awake, good for repeat smaller vols.
10-100ul

Vena Cava (non survival)
Anesthesia required, reliable large vols 1.6-2.5cc

Tail Vein
Performed Awake, smaller vols.

Tail Nick
Performed Awake, small vols.
10-20ul

Dorsal Pedal vein
Performed Awake, good for repeat smaller vols.
Blood collection

- Survival: Mandibular/facial, Tail nick, Saphenous
- Get training if unfamiliar
- Rapid, minimal risk to animal, anesthesia not required.
- Repeat sampling is possible.
- Ensure that bleeding stops and don’t take too much.
Advantages:

1. No Anesthesia required... although can be used
2. Good volume – 150 ul +
3. Repeat samples within day
   • alternate sides
Blood collection: retro-orbital

- Acceptable on awake animals only when scientifically justified and must be a **skilled operator** …get trained
  - Not recommended by NCI-Frederick ACUC due to complications risk
- Mouse has a *large* peri-orbital venous sinus
- Medial (usually) canthus
- Consider local or general anesthetic
- Microhematocrit tubes 75-200 μl
- Do not touch or damage the eye
- Minimum of 10 days for recovery
  - Monitor post collection and report squinting or bulging
- High throughput is possible by a skilled handler

Terminal Blood collection in conjunction with euthanasia

- Anesthesia required prior to exsanguination
- Get trained if planning on performing
  - Retro-orbital
  - Cardiac puncture
  - Posterior vena cava
  - Axillary vessels
Other sample collections

- CSF (8-25 μl from anesthetized mouse)
- Bile (chronic collection surgery prep)
- Bone marrow
- Peritoneal fluid
- Other sample collection

- LASP staff are happy to help you with development of procedures, just ask
Anesthesia and Analgesia
Anesthesia

- **General Points**
  - Preparation is key
  - Hypothermia
  - Monitoring/Depth assessment

- **Injectable anesthetics**
  - Ketamine and Xylazine
    - Most common
  - Tribromoethanol (Avertin)
    - Not recommended/consider alternatives

- **Inhalation anesthesia**
  - Isoflurane (1-5% - usually 2% for maintenance)

- **Local anesthesia**: 
  - Bupivacaine or Lidocaine
  - Use it, it works really well and is reasonably safe
Ketamine and ketamine combinations

- Ketamine produces immobility, analgesia, and amnesia
- Should be combined with a tranquilizer (or other drug) to produce balanced anesthesia (e.g. xylazine)
- Useful starting dose for mice is 100 mg/kg ketamine + 10 mg/kg xylazine
- Ketamine is **Controlled Substance (Schedule III)**
  - Bethesda: DVR Pharmacy in Bldg 14, NIH Policy
    - Training
    - Lock box
    - Every administration: Date, ID, Amount, Signatures
    - Audit occurs
Isoflurane gas

- Machine with precision vaporizer
- Induction chamber
- Maintain with mask or intubation
- Safe, rapid induction and recovery
- Requires scavenging
Analgesia – pain relieving drugs

- Local analgesia – bupivacaine – it works great to relieve short term pain
- Opioid analgesia – buprenorphine, buprenorphine SR (schedule III)
- NSAID – Carprofen, Meloxicam
- Consider multimodal approach
- Recommendations guided by anticipated degree of pain
  - See ACUC guidelines or contact vets
  - For surgery recommend injectable analgesics
- Research effects:
  - unrelieved pain/distress may outweigh temporary side effects of analgesics
Pain in Rodents

- Do rodents feel pain?
- Can we tell if a rodent is feeling pain?
- Will pain relieving drugs affect our research outcomes?
- Will not treating pain affect our research outcomes?
- Potential unwanted variables
  - Pain relieving drug?
  - Pain?
Pain and Distress in Cancer Studies

- The NIH, the ACUC and the public expects (ethically and legally) the most humane methods should be utilized to achieve scientific objectives
  - The three Rs – refine, reduce and replace
  - Minimize unrelieved pain/distress
  - Use analgesics and anesthetics
  - Assume pain or distress in humans = pain or distress in other animals
- Pain or distress may be due to a numerous study induced events - chemo treatments, surgery, irradiation, etc.
- Required scientific justification for procedures that are expected to cause pain or distress
- If you are not sure please ask
Categorizing Pain and Distress

- USDA Pain categories C, D and E (or 1, 2, & 3)
  - Hard to fit everything in a box … but we are trying
- C/1 – No more than momentary or transient pain/distress.
  - e.g. injections/bleeding (multiple??)
  - What is momentary? (NIH OACU defines it as “seconds with low intensity” however, in practice a less strict definition is applied by the NCI ACUC)
- D/2 – Relieved momentary pain/distress
  - e.g. analgesia post surgery (requires scientific justification)
- E/3 – More than momentary pain/distress that is NOT relieved by appropriate methods
  - e.g. no analgesia post surgery, moribund end points, cachexia, seizures
  - ACUC requires scientific justification
- Please note: there are no penalties for accurately categorizing the study as D or E
## Pain category and the NCI ACUC: Examples

- Bethesda and Frederick ACUC have different guidelines
- Bethesda: coming soon...
- Frederick: [https://ncifrederick.cancer.gov/Lasp/Acuc/Frederick/Media/Documents/ACUC7.pdf](https://ncifrederick.cancer.gov/Lasp/Acuc/Frederick/Media/Documents/ACUC7.pdf)

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Cat C</th>
<th>Cat D</th>
<th>Cat E</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQ tumor size diameter (mm)</td>
<td>&lt;20mm &amp; appropriate end points</td>
<td>=20mm and euthanize or treat with analgesics</td>
<td>&gt;20mm</td>
</tr>
<tr>
<td></td>
<td>(Bethesda only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>N/A</td>
<td>With appropriate analgesia</td>
<td>Without appropriate analgesia</td>
</tr>
<tr>
<td>Animal identification and Breeding</td>
<td>Standard ID and Breeding</td>
<td>If &gt; 21 days and need to clip tail with analgesia/anesthesia</td>
<td></td>
</tr>
<tr>
<td>Imaging study</td>
<td>Anesthesia long term studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious disease study</td>
<td>Benign infections/study ends prior to clinical signs</td>
<td>Euthanized at humane endpoints +/- analgesia</td>
<td>Moribund endpoint</td>
</tr>
</tbody>
</table>
Identifying pain

- Difficult ... impossible in some cases
  - Mice are small, nocturnal, vocalize at ultrasonic frequencies, mask pain
  - Variability in observers
  - Variability in strains and sex to pain or response to analgesia
- Get trained: know normal vs abnormal behavior
- Use a scoring system
- Assume pain or distress in humans = pain or distress in other animals
Potential signs of pain or distress

- Early signs are subtle – know your mice
- Decreased food and water consumption – how do you measure this?
- Weight loss – bones prominent, body condition score, frequency
- Dehydration – skin tenting, sunken eyes
- Changed facial expression*
- Nesting and burrowing behavior reduced*
- Isolation/hiding
- Self-mutilation
- Rapid, Open mouth, Abdominal breathing
- Running around in the cage “like normal” doesn’t mean it isn’t in pain


https://www.humane-endpoints.info/en/mouse/nutrition
Euthanasia & end points
Endpoints – experimental or humane

- Both described in your protocol
- Experimental: Defined intervention point:
  - Euthanized at a set time point or after a predetermined event
    - e.g. 10 weeks post injection of drug
    - Sometimes the same as humane end point
- Humane: At defined set of clinical parameters
  - Should be prior to the moribund state (unless cat E)
    - Moribund = in the process of dying
  - Allows for data collection prior to onset of unnecessary pain or distress
  - May be based on more than one parameter
Euthanasia: Rodents > 10 days old

- Do not overcrowd cage
- Recommendation: No more than 10 mice per cage (or 20 pre-weanlings)
- Home cage is best
- Do not mix live animals with dead animals
Using the CO2 euthanasia lid system

- Remove cage filter top
- Put stainless steel lid over cage
- Turn on CO$_2$ gas
- Adjust flow meter to 2 lpm (std cage)
  - Different size container $\rightarrow$ different rate – ask for advice
- Increase rate (10 lpm) once unresponsive for 2 mins
- Wait 60s – no movement
Alternative for neonates: Isoflurane anesthetic

- \( \geq \) 20 minute exposure to Isoflurane in a jar will kill neonates
- Downdraft table, bell jar, and Isoflurane needed
- No direct contact
ACUC Guidelines

Frederick
https://ncifrederick.cancer.gov/Lasp/Acuc/Frederick/GuidelinesFnl.aspx

Bethesda
LASP SOPs

Final Messages

- Problems with techniques may never be detected unless you are well-trained and committed to excellence.
- Monitoring during and after procedures is essential.
  - Repeated monitoring:
    - determination of progression
    - intervention before it’s too late
Sign-up for hands on training

• Bethesda Campus:
  • Email completed form to:
    • Dr. Melody Roelke-Parker, melodyr@mail.nih.gov

• Frederick Campus:
  • Email:
    • Dr. Jatinder Gulani, Jatinder.gulani@nih.gov
    • Dr. Gillian Braden, gillian.braden-weiss@nih.gov
Acknowledgements:

Bethesda
LASP staff:
Devorah Gallardo
Andrew Okoth
Gail McMullen
Sam Kimani
Melody Roelke-Parker
Bob Hoyt
Josh Kramer
Larry Cook

NCI staff:
Wendy Dubois
Juan Yin (Ivy)
Christine Hollander
Fabian Mueller

Frederick
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Gillian Braden
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Blayre Montague