Schmerzbehandlung und Anaesthesie bei Labormäusen:
Trends und Probleme aus der Praxis

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PD Dr. med. vet. DipECLAM
Goals

Anesthesia

- species-specific problems in mouse anesthesia
- types of anesthesia
  - injection anesthesia
  - inhalation anesthesia
  - examples, problems
  - optimized protocols

Pain therapy

- decide on pain therapy
- drugs, side effects
- dosages
  - intensive post-operative care in mice
Species-specific problems in mouse anesthesia

the mouse
- 18 – 50 g body weight
- hypothermia
- access to arteries, veins
- injection side
  i.p., s.c.
- monitoring
  blood pressure
  heart rate, ECG
  pulsoximetry
  blood gases and acid base balance
- intervention
  cardiac arrest
  respiratory depression

the drugs
- feasibility
  Propofol®, diazepam, di-ethyl-ether, …
- availability
  InnovarVet® > off the market
  Hypnorm® > narcotics laws, import from UK
  Halothane > off the market?
  Metofane® > import from Australia
- regulations for protection of the personnel
  secure gas scavenging
- legal restrictions from welfare concerns
  Avertin®, di-ethyl-ether, …
Species-specific problems in mouse anesthesia
Zeller, W., Meier, G., Bürki, K., Panoussis, B.: Adverse effects of tribromoethanol as used in the production of transgenic mice. Laboratory animals, 1998, 32, 407-13


Lieggi, C.C., Artwohl, J.E.Leszczynski, J.K., Rodriguez, N.A., Fickbohm, B.L., Fortman, J.D.: Efficacy and safety of stored and newly prepared tribromoethanol in ICR mice. Contemporary topics in laboratory animal science, 2005, 44/1, 17-22


Types of anesthesia: route of application

**Injection**
- ease of application
  - i.p.
  - s.c.
- special equipment: none
- environmental impact: no
- cheap?
- not controllable

**Inhalation**
- application
  - induction chamber
  - face mask
- special equipment
- environmental impact?
- dangerous for personnel?
- expensive?
- controllable

Mono-anesthetics
- e.g. pentobarbital

Combination of dissoziative + alpha-two agonist
- e.g. ketamin + xylazine

Combination of opioid + alpha-two agonist
- e.g. fentanyl + medetomidine

Isoflurane
Sevoflurane
Desflurane

Halothane
Enflurane
Methoxyflurane
Di-ethyl-ether
Injection anesthesia

Combination of substances

synergistic action
- adverse effects decreased
- desired effects increased
- analgesia strengthened

examples
- neurolept-analgesia
  - fentanyl + *fluanison* (Hypnorm®) + medetomidine
  - fentanyl and medetomidine can be antagonized
  - fentanyl + midazolam + medetomidine
  - all components can be antagonized
    [from J. Henke, Munich]
- ketamin + xylazine (Rompun®) [+ acepromazine]
<table>
<thead>
<tr>
<th>Substances</th>
<th>Range of dosages [mg/kg]</th>
<th>Substance class</th>
<th>Availability</th>
<th>Handling</th>
<th>Lack of toxicity</th>
<th>Efficacy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mono-anesthetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>30 – 90</td>
<td>Barbiturate</td>
<td>–</td>
<td>n</td>
<td>+</td>
<td>narrow safety margin</td>
<td>analgesia insufficient, long sleeping time</td>
</tr>
<tr>
<td>α-Chloralose</td>
<td>114</td>
<td>–</td>
<td>–</td>
<td>difficult to dissolve in saline</td>
<td>–</td>
<td>matter of controversy</td>
<td>–</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>60 - 400</td>
<td>–</td>
<td>n</td>
<td>–</td>
<td>not available in sterile form, light sensitive, air sensitive</td>
<td>–</td>
<td>high mortality, intestinal complications, out of use today</td>
</tr>
<tr>
<td>Etomidate</td>
<td>23.7 - 33</td>
<td>Non-barbiturate hypnotic</td>
<td>–</td>
<td>c</td>
<td>+</td>
<td>–</td>
<td>tissue irritant</td>
</tr>
<tr>
<td><strong>Combinations of anesthetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine + Xylazine</td>
<td>50 – 200</td>
<td>Dissociative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>analgesia, no relaxation</td>
<td>5, 13, 14, 15, 16</td>
</tr>
<tr>
<td>Ketamine + Medetomidine</td>
<td>75</td>
<td>Dissociative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>analgesia, no relaxation</td>
<td>14, 15, 18, 19, 20, 21</td>
</tr>
<tr>
<td>Ketamine + Azaperone</td>
<td>100</td>
<td>Dissociative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>analgesia, no relaxation</td>
<td>16, 17</td>
</tr>
<tr>
<td>Telatamin/ Zolazepam (Telazol™) + Xylazine</td>
<td>7.5 – 100</td>
<td>Dissociative/ Benzodiazepine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Telazol alone: only immobilization</td>
<td>5, 7</td>
</tr>
<tr>
<td>Fentanyl/ Fluaniison (Hypnorm™) + Midazolam or + Diazepam</td>
<td>0.33 – 3.3 [ml/kg]</td>
<td>Opioid/ Butyrophenone</td>
<td>–</td>
<td>n, c</td>
<td>–</td>
<td>precipitates out of solution, difficult to mix with other drugs, storage of mixtures not possible</td>
<td>neuroleptanalgesia</td>
</tr>
<tr>
<td>Fentanyl/ droperidol (Innovar-Vet™) + Diazepam</td>
<td>0.0001 – 0.001 [ml/g]</td>
<td>Opioid / Butyrophenone</td>
<td>–</td>
<td>n, c</td>
<td>+</td>
<td>+</td>
<td>sedation</td>
</tr>
<tr>
<td>Fentanyl + Metomidate</td>
<td>0.06 - 0.08</td>
<td>Opioid Non-barbiturate hypnotic</td>
<td>–</td>
<td>n, c</td>
<td>+</td>
<td>+</td>
<td>sedation</td>
</tr>
<tr>
<td>Fentanyl + Etoridate</td>
<td>0.08</td>
<td>Opioid Non-barbiturate hypnotic</td>
<td>–</td>
<td>n</td>
<td>+</td>
<td>–</td>
<td>tissue irritant; high mortality?</td>
</tr>
<tr>
<td>Carfentanyl + Etoridate</td>
<td>0.003</td>
<td>Opioid Non-barbiturate hypnotic</td>
<td>–</td>
<td>n</td>
<td>+</td>
<td>–</td>
<td>side effects: excitations, muscle spasms</td>
</tr>
</tbody>
</table>

*a* = commercially available; n = legal restrictions in some countries (narcotics act); c = not available in some countries; o = off the market.

*b* = available in sterile form, easy to dilute or mix with other drugs, chemically stable, can be stored.

c = Toxicity defined as mortality, tissue irritancy, other side effects. Safety margin = effective dose vs. toxic dose. * = no relevant experimental data in the mouse were found.

d = Efficacy defined in terms of the 3 components of anesthesia: analgesia, hypnosis, and muscle relaxation.

from Arras et al. Comp Med 2001
Sensitivity to injectable anesthetics influenced by...

strain, sex, age


circadian rhythm, health, pregnancy, socio-physiological conditions, adaptation


housing conditions

Dairman, W., Balasza, T. 1970. Comparison of liver microsome enzyme systems and barbiturate sleeping times in rats caged individually or communally. Biochemical Pharmacology 19:951-955

genetic modification

• Takei, T., Saegusa, H., Zong, S., Murakoshi, T., Makita, K., Tanabe, T. 2003. Increased sensitivity to halothane but decreased sensitivity to propofol in mice lacking the N-type Ca channel. Neuroscience letters 350: 41-45
Percentage of mice reaching surgical tolerance and survival rate

n=20
Stock Hsd:NMRI male
age 3-6 months

Ket = ketamin
Xyl = xylazine
Ace = acepromazine
Aza = azaperone
Tel = Telazol®
(tiletamine + zolazepam)
Med = medetomidine

Ket 100 Xyl 20
Ket 150 Xyl 30
Ket 100 Xyl 20 Ace 3
Ket 100 Xyl 20 Aza 5
Tel 80 Xyl 20
Ket 100 Aza 80
Ket 100 Med 1
Ket 100 Med 5

surgical tolerance
survival rate
dosages in mg/kg bodyweight
**Time of surgical tolerance and immobilization**

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>Dosage</th>
<th>Surgical Tolerance</th>
<th>Immobilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Ket 100 Xyl 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Ket 150 Xyl 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Ket 100 Xyl 20 Ace 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>Ket 100 Xyl 20 Aza 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>Tel 80 Xyl 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>Ket 100 Aza 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>360</td>
<td>Ket 100 Med 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>420</td>
<td>Ket 100 Med 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Dosages in mg/kg bodyweight**

- Ket = ketamin
- Xyl = xylazine
- Ace = acepromazine
- Aza = azaperone
- Tel = Telazol® (tiletamine + zolazepam)
- Med = medetomidine

Stock Hsd:NMRI male age 3-6 month

n=20
Summary

**ketamin + xylazine**
- surgical tolerance: 25 min
- immobilization: approx. 2 hours

**ketamin + xylazine + acepromazine**
- surgical tolerance: 50 min
- immobilization approx. 2 hours
- low death rate
- acceptable safety margin

for results and details, see Arras et al. Comp Med, 2001
Improved ketamin xylazine anesthesia

Surgical tolerance: 50 min
Restraint: 120 min

**recommended dosage**

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamin</td>
<td>65 mg/kg bodyweight</td>
</tr>
<tr>
<td>Xylazine</td>
<td>13 mg/kg bodyweight</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>2 mg/kg/bodyweight</td>
</tr>
</tbody>
</table>

for results and details, see Arras et al. Comp Med, 2001
### Example for neuroleptanalgesia with antagonization

**Dosierung VAA Kleinsäuger (in mg/kg)**

Julia Henke, Wolf Erhardt

<table>
<thead>
<tr>
<th></th>
<th>Kaninchen i.m.</th>
<th>Meerschweinchen i.m.</th>
<th>Chinchilla i.m.</th>
<th>Ratte i.m.</th>
<th>Hamster i.p.</th>
<th>Gerbil s.c.</th>
<th>Maus i.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fentanyl</strong></td>
<td>0,02</td>
<td>0,025</td>
<td>0,02</td>
<td>0,005</td>
<td>0,033</td>
<td>0,03</td>
<td>0,05</td>
</tr>
<tr>
<td><strong>Midazolam</strong></td>
<td>1,0</td>
<td>1,0</td>
<td>1,0</td>
<td>2,0</td>
<td>3,3</td>
<td>7,5</td>
<td>5,0</td>
</tr>
<tr>
<td><strong>Medetomidin</strong></td>
<td>0,2</td>
<td>0,2</td>
<td>0,05</td>
<td>0,15</td>
<td>0,33</td>
<td>0,15</td>
<td>0,5</td>
</tr>
<tr>
<td><strong>Naloxon</strong></td>
<td>0,03</td>
<td>0,03</td>
<td>0,05</td>
<td>0,12</td>
<td>0,8</td>
<td>0,5</td>
<td>1,2</td>
</tr>
<tr>
<td><strong>Flumazenil</strong></td>
<td>0,1</td>
<td>0,1</td>
<td>0,1</td>
<td>0,2</td>
<td>0,33</td>
<td>0,4</td>
<td>0,5</td>
</tr>
<tr>
<td><strong>Atipamezol</strong></td>
<td>1,0</td>
<td>1,0</td>
<td>0,5</td>
<td>0,75</td>
<td>1,7</td>
<td>0,375</td>
<td>2,5</td>
</tr>
</tbody>
</table>

Antagonisierung routinemäßig s.c., in Notfällen i.v. mit halber Dosis
Statt Midazolam auch Climazolam, nicht Diazepam, statt Flumazenil auch Sarmazenil
Bei jungen und leichten Meerschw. evtl. auch Flumazenil weglassen
Bei Bedarf 1/3 der Ausgangsdosis nachdosieren
Zwergkaninchen nur 2/3 der Dosis

aus: Erhardt, Henke, Lendl: Narkosenotfälle, ENKE 2002
Short intraperitoneal injection anesthesia

Surgical tolerance: 15 min
Restraint: 30 min

- Propofol 75 mg/kg bodyweight
- Medetomidine 1 mg/kg bodyweight
- Fentanyl 0.2 mg/kg bodyweight

from
Alves, HC, Valentim, AM, Olsson, IAS, Antunes, LM
Laboratory Animals, 2009, 43: 27-33
General drawbacks

Injection anesthesia in mice
  – narrow safety margin
  – special care: hypothermia

Volatile anesthetics: halothane, isoflurane
  – affect fertility?
  – mutagenic?, teratogenic?
  – hepatotoxicity?
Isoflurane: long-term exposition of staff, health risks from daily work with Isoflurane

- pregnant women should stay away from rooms in which inhalation anaesthesia is performed
- for pregnant women it is prohibited to work in rooms, in which halothane is used

*Consider specific regulations if working with volatile anesthetics!!!*

Recommended specific literature, job security:
Umgang mit Anästhesiegasen; Gefährdung, Schutzmassnahmen
Schweizerische Unfallversicherungsanstalt, Abteilung Arbeitsmedizin, Postfach, 6002 Luzern
Tel.: 041 419 51 11, Fax 041 419 58 28
Contemporary anesthesia protocol for short- and long-term interventions

Sevoflurane, 4% - 8% via face mask
  death rate: <1%
  costs: < 10 CHF/ h anesthesia
Optimization of inhalation anesthesia in laboratory mice (balanced anesthesia)

Injection of fentanyl 0.4 mg/kg + midazolam 4 mg/kg, s.c., at 10 to 15 minutes prior to induction with volatile anesthetics, e.g. sevoflurane (3.5%), or isoflurane

Injection of ketamin 30 mg/kg body weight subcutaneously, at 10 to 15 minutes prior to induction with volatile anesthetics, e.g. sevoflurane (4.9%), or isoflurane
How to decide on pain therapy

experimental design
  – degree of pain
  – duration of pain

animal species
  – application route
  – frequency of application

interference with the experiment

select an analgesic drug
# Types of analgesic drugs

## Opioids
(acting mainly on the central nervous system)
severe pain
availability?

**side effects**
- respiratory depression
- obstipation
- rat: pica behavior if overdosed, = rat eats bedding, papers, towels etc.!
- Buprenorphin: behavioural aberrations

## NSAID
(peripheral action)
anti-inflammatory
anti-pyrogenic

**side effects**
- inhibition of platelet aggregation!
- long-term application: kidney function decreased
- stomach ulceration
- bleeding in the gastrointestinal tract
Trends in rodent pain therapy

Table from Claire A. Richardson and Paul A. Flecknell: Anaesthesia and post-operative Analgesia following experimental surgery in laboratory rodents: Are we making progress? ATLA 33, p. 119-127, 2005

<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Suggested analgesic dose rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.01-0.05mg/kg s.c., 6-12 hourly</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>2.0mg/kg s.c., 4 hourly</td>
</tr>
<tr>
<td>Morphine</td>
<td>2-5mg/kg s.c., 4 hourly</td>
</tr>
<tr>
<td>Pethidine</td>
<td>10-20mg/kg i.m., s.c., 2-3 hourly</td>
</tr>
<tr>
<td>Carprofen</td>
<td>5mg/kg s.c., ? daily</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1-2mg/kg s.c., or 4mg/kg per os ? daily</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5mg/kg s.c., ? daily</td>
</tr>
</tbody>
</table>

Dose rates are based largely on uncontrolled clinical trials and a limited range of procedures, and so are likely to be subject to revision. Whenever possible, a pain scoring scheme should be used, so that the dose rates can be adjusted according to an animal’s response.

i.m. = intramuscular injection; per os = by mouth; s.c. = subcutaneous injection.
## Dosages of NSAID for mice, taken from literature

<table>
<thead>
<tr>
<th>Year</th>
<th>Carprofen</th>
<th>Meloxicam s.c.</th>
<th>Meloxicam Per os</th>
<th>Flunixin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>10 mg/kg s.c.</td>
<td>5 mg/kg s.c.</td>
<td></td>
<td></td>
<td><a href="http://www.ahwla.org.uk/site/tutorials/RP/RP11-Where.html">www.ahwla.org.uk/site/tutorials/RP/RP11-Where.html</a></td>
</tr>
<tr>
<td>2005</td>
<td>10 mg/kg s.c.</td>
<td>5 mg/kg s.c.</td>
<td>? daily</td>
<td></td>
<td>Claire A. Richardson and Paul A. Flecknell ATLA 33, p. 119-127, 2005</td>
</tr>
<tr>
<td>2000</td>
<td>5 mg/kg s.c.</td>
<td>?</td>
<td>?</td>
<td>2.5 mg/kg s.c.;</td>
<td>Pain Management in Animals by P. Flecknell and A. Waterman-Pearson, Harcourt Intern., London, 2000</td>
</tr>
<tr>
<td></td>
<td>or by mouth daily</td>
<td></td>
<td>?</td>
<td>? 12-24 hourly</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>-</td>
<td></td>
<td></td>
<td>2.5 mg/kg s.c., i.m.;</td>
<td>Laboratory animal Anaesthesia, by Paul A. Flecknell, Harcourt International, London, 2nd ed. 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>? 12 hourly</td>
<td></td>
</tr>
</tbody>
</table>
Intensive care in mice after major surgery

prior to surgery
  – provide high energy food (e.g. solid drink®-Energy) and glucose 15% in the water bottle

during anesthesia/surgery
  – after induction of anesthesia: injection of 1 mL NaCl 0.9% i.p.
  – induction of post-operative analgesia at 20-30 minutes before anesthesia is finished, e.g. flunixin 5-7 mg/kg body weight s.c. or buprenorphine 0.1 mg/kg body weight s.c.
  – prevent hypothermia during anesthesia and in the post-operative phase
  – put mice back in their home cage, not in a new territory
Intensive care in mice after major surgery

after surgery (for up to 7 days)

– heating pad underneath the cage
– oxygen supply in the cage
– in 12-hours intervals:
  flunixin 5 mg/kg BW s.c. (or buprenorphin 0.1 mg/kg BW s.c.)
  0.3 mL NaCl 0.9% s.c. and 0.3 mL glucose 5% s.c.
– provide high energy food, and food pellets; glucose 15%, in the drinking bottle and in dishes on the cage ground
– 1-2 times per day: check body weight, food and water consumption, and the animals outer appearance and moving behavior

Schuler, B. et al., submitted
Fluid therapy

Ringer-Lactat or NaCl 0.9% (37°C)

in general
10 ml/kg/h i.v.

rat
5-10 ml i.p. during laparatomy

mouse
0.5-1.0 ml i.p. during laparatomy
0.5-0.7 ml s.c. after surgery in 12 hour-intervals
Thank you for your attention