Analgesia and Anaesthesia in animal tests

Prof Mario Giorgi ChemD specPharmacol

Dept Veterinary Sciences, University of Pisa, Italy
Pain

What is pain?

How can I assess it?

Is a pain test producing the same pain in different animal species?

Have different animal same sensitivity to pain?

How can I assess that an test is painful?

Is it mandatory that any test model has an analgesic drug included?
Pharmacokinetics and pharmacodynamics of zolpidem after oral administration of a single dose in dogs

Mario Giorgi, ChemD, MSPharmacol; Diego Angel Portela, DVM, PhD; Gloria Breghi, DVM; Angela Briganti, DVM, PhD

Objective—To evaluate the pharmacokinetics and pharmacodynamics of zolpidem after oral administration of a single dose (0.15 or 0.50 mg/kg) and assess any associated antianxiety and sedative effects in dogs.

Animals—8 clinically normal sexually intact male dogs of various breeds.

Procedures—Dogs were assigned to 2 groups (4 dogs/group) and administered zolpidem orally once at a dose of 0.15 or 0.50 mg/kg in a crossover study; each dog received the other treatment once after an interval of 1 week. Blood samples were collected before and at intervals during the 24-hour period following dose administration. For each time point, plasma zolpidem concentration was evaluated via a validated method of high-performance liquid chromatography coupled with fluorescence detection, and pharmacodynamics were assessed via subjective assessments of sedation and level of agitation and selected clinical variables.

Results—The pharmacokinetic profile of zolpidem in dogs was dose dependent, and the plasma drug concentrations attained were lower than those for humans administered equivalent doses. The lower dose did not result in any clinical or adverse effects, but the higher dose generated paradoxical CNS stimulation of approximately 1 hour’s duration and a subsequent short phase of mild sedation. This sedation phase was not considered to be of clinical relevance. The desired clinical effects were not evident at plasma zolpidem concentrations ≤ 30 ng/mL, and the minimal plasma concentration that induced adverse effects was 60 ng/mL.

Conclusions and Clinical Relevance—Results indicated that zolpidem is not a suitable drug for inducing sedation in dogs. (Am J Vet Res 2012;73:1650–1656)
The most sold hypnotic drug over the world
Few side effects
Unique PK profile → customization of treatment for various types of insomnia.
Mechanism of action on GABA$_A$

- GABA$_A$ in the brain has very strong hypnotic properties.
- GABA$_A$ in the spine and peripheral tissues has very weak anxiolytic, myorelaxant, and anticonvulsant properties.

BZDs (Benzodiazepines) are involved in these actions.
Why ZP in Med Vet?

THERE IS A NEED FOR SAFE AND RAPID REDUCTION IN RESPONSIVENESS TO ENVIRONMENTAL STIMULI AND INITIATION OF SLEEP, WITH RELATIVELY SHORT DURATION OF ACTION AND RAPID RECOVERY

RAPID ONSET AND ACTION IN SEVERE PHOBIC STATES
# CATEGORIES OF INVASIVENESS IN ANIMAL EXPERIMENTS

**“PAIN CATEGORIES”**

<table>
<thead>
<tr>
<th>Pain Category</th>
<th>Type of Experiments / Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C</strong></td>
<td>Experiments / Procedures causing <em>little discomfort or stress</em></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Experiments / Procedures causing <em>moderate to severe distress or pain</em> using anesthesia and / or painkiller</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>Experiments / Procedures causing <em>prolonged or severe clinical distress or pain</em> without the use of anesthesia and / or painkiller</td>
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</table>
Pain Category C

• **Category C procedures include:**

Experiments **causing minor stress of short duration**
Level C procedures should not cause significant changes in the animal’s appearance,

• **Category C procedures include:**

  Injection of material in amounts that will not cause adverse reactions
  Acute non-survival studies in which the animals are completely anaesthetized and do not regain consciousness
  Approved methods of euthanasia following rapid unconsciousness
  Short periods of food and/or water deprivation equivalent to periods of abstinence in nature
  Cannulation or catheterization of blood vessels or body cavities under anesthesia
  Minor surgical procedures under anesthesia - biopsies, laparoscopy
  Short periods of skillful restraint beyond that for simple observation or examination
  Blood sampling
Pain Category D

Category D procedures include:

Experiments causing *moderate to severe distress or pain* using anesthesia and / or painkiller
Level D procedures should not cause prolonged or severe clinical distress

Category D procedures include:
Major surgical procedures conducted under anesthesia with subsequent recovery
Prolonged (several hours or more) periods of physical restraint
Induction of behavioral stress - like maternal deprivation, aggression, predator-prey interactions
Procedures causing severe, persistent or irreversible disruption of sensorimotor organization
Pain Category E

Category E procedures include:
Experiments causing prolonged or severe clinical distress and / or pain without the use anesthesia and / or painkiller

✓ Procedures inflicting severe pain near, at, or above the pain tolerance threshold of not anesthetized, conscious animals

✓ Not confined to surgical procedures, but may include exposure to noxious agents or those having unknown effects

✓ Exposure to drugs or chemicals at levels that may impair physiological systems and cause death, severe pain, or extreme distress

✓ New biomedical experiments having a high degree of invasiveness

✓ Behavioral studies having unknown degree of distress

✓ Muscle relaxant or paralytic drug use without anesthetics

✓ Burn or trauma infliction on not anesthetized animals

✓ Toxicity testing and experimentally-induced infectious disease studies that have death as the endpoint
Pain definition

The American Academy of Pain Medicine defines pain as:

“An unpleasant sensation (that can range from mild, localized discomfort to agony) and emotional response to that sensation”.
Distress definition
(Merriam – Webster)

Synonyms: **distress**, **suffering**, **misery**, **agony**
meaning the state of being in great trouble.

- **Distress** implies an external and usually **temporary** cause of great physical or mental strain and stress
- **Suffering** involves **conscious endurance** of pain or distress
- **Misery** stresses the **unhappiness**, poverty
- **Agony** means **intense feelings of suffering**; acute mental or physical pain
Assessment of pain or distress may be based on many different criteria

- Decreased activity
  - Abnormal postures, hunched back, muscle flaccidity or rigidity
  - Poor grooming
  - Decreased food or water consumption
  - Decreased fecal or urine output
- Weight loss (generally 20-25% of baseline), failure to grow, or loss of body condition (cachexia)
  - Dehydration
  - Decrease or increase in body temperature
  - Decrease or increase in pulse or respiratory rate
- Physical response when touched
  (withdrawal, lameness, abnormal aggression, vocalizing, abdominal splinting, increase in pulse or respiration)
  - Teeth grinding
- Self-aggression
- Inflammation
- Photophobia
- Vomiting or diarrhea
- Objective criteria of organ failure demonstrated by: hematological or blood chemistry values, imaging, biopsy, or gross dysfunction
Ethical Considerations

• An important ethical principle of animal use in biomedical research is that alternatives to live animals should be used whenever possible.
• Documentation of a search for alternatives and an explanation for why these alternatives were not found to be suitable or how alternatives were incorporated into the experimental design is a mandatory requirement.
• Exploring alternatives to animal use may be accomplished by using the three Rs; Replacement, Reduction, and Refinement
Types of Pain

Acute Pain
- Occurs immediately after a stimulus is received
- Severity can vary
- Responds well to treatment
- Subsides once stimulus is removed

Chronic Pain
- Persists well past initial stimulus (3-6 months)
- Severity can vary
- May or may not respond well to treatment; may require a “multi-modal” approach
- Can result in allodynia, hyperalgesia, and opioid tolerance
Physiology of Pain

There are four distinct processes involved in nociception which can be modulated by analgesics:

– **Transduction** – translation of the noxious stimulus into electrical activity at the peripheral nociceptor

– **Transmission** – the propagation of nerve impulses through the nervous system

– **Perception** – the final conscious subjective and emotional experience of pain

– **Modulation** – modification of nociceptive transmission by inhibition of the spinal dorsal horn cells by endorphins
Actions of Analgesics on Pain Processes

Transduction:
- Can be blocked by local anesthetics by injection either at the site of injury/incision or intravenously
- Can be decreased by use of NSAIDs which decrease the production of prostaglandins at the site of injury

Transmission:
- Can be prevented by local anesthetics by injection along peripheral nerves, at nerve plexus, or in the epidural or subarachnoid spaces

Perception:
- Altered by use of general anesthetics or systemic injection of opioids and/or alpha\textsubscript{2}-agonists

Modulation:
- Can be augmented by injection of local anesthetics or alpha\textsubscript{2}-adrenergic agonists; gabapentin may also effect modulation
Actions of Analgesics on Pain Processes

- **Pre-emptive analgesia**: giving analgesics prior to the noxious stimulus (surgery)
  - By blocking or inhibiting the nociceptive process before it begins, hypersensitivity is prevented
  - May decrease the amount of anesthesia and post-operative analgesia needed

- **Multimodal or “balanced” analgesia**: using a combination of analgesics which will impact more than one portion of the nociceptive process
  - For example: buprenorphine and meloxicam pre-surgically, lidocaine block used prior to incision, and bupivacaine splash prior to closing incision
Analgesics

• Divided into five main classes based on mode of action
  – Opioids
  – Non-steroidal anti-inflammatory drugs
  – Local anesthetics
  – Alpha$_2$-adrenoceptor agonists
  – Miscellaneous drugs
Analgesics - Opioids

- Bind to opioid receptor sites within CNS (mostly μ but also κ)
- Are agonists, partial agonists or mixed agonists-antagonists
- Are controlled substances requiring special licenses and documentation of usage
Opioids

- Agonists – include morphine and fentanyl
  - Potent opioid analgesics
  - Have more serious potential side effects than the mixed agonist/antagonists: respiratory depression, bradycardia, vomiting, constipation
  - Can be used in a continuous infusion during anesthesia
  - Combined with tranquilizers for neuroleptanalgesic balanced anesthesia
  - Can be administered intravenously, intramuscularly, via transdermal patches, and epidurally +/- local anesthetics
  - Can be reversed with naloxone
Opioids

• Mixed agonist-antagonist – includes butorphanol
  – Have agonist or partial agonist activity at one or more opioid receptors and the ability to antagonize the effects of a full agonist at one or more opioid receptor
  – Butorphanol is a mu antagonist and kappa agonist
  – Butorphanol isn’t routinely used for analgesia currently due to it’s dosing frequency
  – Less respiratory depression than full agonists
  – Can be used post-operatively to reverse the narcosis of fentanyl while still providing analgesia
  – Has a “ceiling” effect, at which point increased doses won’t have any further effect
Opioids

- Partial Agonist – includes buprenorphine
  - Has both agonist and antagonist activity at the mu receptor
  - Can be used to reverse pure mu agonists
  - Buprenorphine has a prolonged duration of action (relatively)
  - Also potential for ceiling effect
Other classification

OPIOIDS

Pure (acute)

Atipycal (chronic)
Phase I metabolism

Main pathways

CYP2D6

TRAMADOL

M1 conjugates

M5 conjugates

M4 conjugates

CYP3A4, CYP2B6

M2

M3

M4

M5

M1

M2

M3

M4

M5
Dynamic (summary)

- S-enantiomer 5-HT uptake inhibition: 20%
- Activity
- μ-agonist 40%
- R-enantiomer 40%
- NE uptake inhibition

Graph showing:
- % Analgesia vs. Dose of opioid antagonist
- Tramadol, Morphine, Codeine
Pharmacokinetic studies

- M1 high plasma concentrations
  - Pypendop & Ilkiw., 2008

- M1 poor plasma concentrations
  - Kukanich & Papich, 2004; Giorgi et al., 2008, 2009a,b,c
  - de Sousa et al., 2008
  - Giorgi et al., 2007, 2010; Shilo et al., 2008
  - Souza et al., 2009
  - Souza and Cox 2011
  - Elgazali et al., 2008
  - Giorgi & Andreoni, 2010
Pain interpreted and processed

Ascending pathways

Descending pathways

Pain stimuli

Peripheral nociceptors
Pain stimuli are interpreted and processed by the brain through ascending and descending pathways. Pain stimuli activate peripheral nociceptors, which then release exciting neurotransmitters. However, there is a reduction in the release of these neurotransmitters, leading to a decrease in the perception of pain.
Pharmacokinetics of tramadol and metabolites after injective administrations in dogs

M. Giorgi¹, S. Del Carlo², B. Łebkowska-Wieruszewska³, C.J. Kowalski¹, G. Saccomanni²

¹ Department of Veterinary Clinics, University of Pisa, Via Livornese (lato monte) 1, San Piero a Grado, 56010 Pisa, Italy
² Pharmaceutical Sciences, University of Pisa, Via Bonanno 5, 56126 Pisa, Italy
³ Department of Pharmacology, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland

Pharmacokinetic and urine profile of tramadol and its major metabolites following oral immediate release capsules administration in dogs

M. Giorgi • S. Del Carlo • G. Saccomanni • B. Łebkowska-Wieruszewska • C. J. Kowalski

Characterisation of tramadol, morphine and tapentadol in an acute pain model in Beagle dogs

Babette Kögel, Rolf Terlinden & Johannes Schneider

Grüenthal GmbH, Grüenthal Innovation, Global Preclinical R&D, Aachen, Germany

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RESEARCH PAPER

Effect of grapefruit juice

Gior

¹Veterinary Teaching Hospital (lato monte) 1 San Piero a Grado, 56126 Pisa, Italy; ¡Institute of Medical Science, Daejeon 305-764, South Korea
²Correspondence: M. Giorgi, Veterinary Teaching Hospital 24 h, Department of Veterinary Clinics, University of Pisa, Via Livornese (lato monte) 1 San Piero a Grado, 56010 Pisa, Italy. E-mail: mgior@vet.unipi.it

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Non-steroidal Anti-inflammatory Drugs (NSAIDs)

- NSAIDs are weak organic acids with anti-inflammatory, analgesic, and antipyretic properties
- Inhibit prostaglandin production by inhibiting COX enzymes
- Are either non-selective (inhibits both COX iso-enzymes) or selective for COX-2
- Non-selective NSAIDs have more serious side effects (gastric ulceration and renal toxicity)
- Decreased renal blood flow during anesthesia makes kidneys more susceptible to toxic effects
- Carprofen and meloxicam are COX-2 selective inhibitors which have a reasonable margin of safety when used pre-operatively
<table>
<thead>
<tr>
<th>Drug related to arachidonic cascade</th>
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<tbody>
<tr>
<td><strong>NSAIDS</strong></td>
</tr>
<tr>
<td>COX 3 (metamizole)</td>
</tr>
<tr>
<td>COX ( \frac{1}{2} ) (meloxicam)</td>
</tr>
<tr>
<td>COX 2 (cimicoxib)</td>
</tr>
<tr>
<td><strong>Correlated molecules</strong></td>
</tr>
<tr>
<td>5LP (tepoxalin)</td>
</tr>
<tr>
<td>SEHIs (pre clinical study)</td>
</tr>
<tr>
<td>EP antagonist (grapiprant)</td>
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Robenacoxib an example

Silber et al., Pharm Res, 2010
Drug $pK_a$ and Drug Distribution
Tepoxalin
(Zubrin®- dog)

Main effects

Poor GI effects

Active metabolite RWJ-20142

Elimination via feces
Pharmacodynamic

Arachidonic acid

Tepoxalin inhibits both COX-1 and COX-2 enzymes as well as 5-lipoxygenase (5-LOX), which is associated with the production of pro-inflammatory and gastrotoxic leukotrienes.

COX-1

COX-2

5-LOX

Prostaglandins (PGD₂, PGE₂, PGE, PG₁₂)

Tromboxanes (TXA₂)

Platelet function

Platelet function

Gastric ulcers

Renal function

Leucotrienes (LTB₄, LTC₄, LTD₄, LTE₄)

Asmatics attacks and muscle contractions

Responsible for gastric protection, renal function and normal platelet function
Arachidonic acid

**Metabolism**

- COX: Drugs targeting prostaglandins, for example: aspirin, ibuprofen, celecoxib
- LOX
- CYP: Epoxygenase, Hydrolase

**Prostaglandin**
- Pain
- Inflammation
- Blood clotting
- Pulmonary hypertension

**Leukotriene C4**
- Drugs targeting leukotrienes, for example, montelukast
- Asthma
- Allergies

**DHETs**
- Cardiovascular disease
- Pain
- Inflammation
- Diabetes
- Kidney disease

**EETs**
- Potential drugs: EET agonist, sEH inhibitor, combined sEH inhibitor and EET agonist
Use of a soluble epoxide hydrolase inhibitor as adjunctive analgesic in a laminitic horse

Alonso G. P. Guzmán1, Christophe Morisseau2, Albert Sole3, João H. N. Soares3, Arzu Ulu4, Hua Dong5, and Bruce D. Hammock6

1Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA, USA
2Department of Entomology and U.C.D. Cancer Center, University of California, Davis CA 95616 USA
3Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, CA, USA

Abstract

A 4-year-old, 500 kg Thoroughbred female horse diagnosed with bilateral forelimb laminitis and oedema on the left forelimb became severely painful and refractory to non-steroidal anti-inflammatory therapy (flunixin meglumine on days 1, 2, 3 and 4, and phenylbutazone on days 5, 6 and 7) alone or in combination with gabapentin (days 6 and 7). Pain scores assessed independently by three individuals with a visual analog scale (VAS, 0 = no pain and 10 = worst possible pain) were 7.5 on day 2, and it increased to 8.5 on day 7. Non-invasive blood pressure monitoring revealed severe hypertension. As euthanasia was being considered for humane reasons as well as technical and financial constraints, a decision was made to add an experimental new drug, per-4-(4-13-trifluoromethylphenyl)-4-oxa-2-oxazolidinone (4-TP), which is an inhibitor of soluble epoxide hydrolase (sEH), to the treatment protocol. Doses and frequency of administration were selected to produce plasma concentrations within the range of 2.5 μM and 30 nM based on the drug potency against sEH. Pain scores decreased sharply and remarkably following sEH inhibition and blood pressure progressively decreased to physiologic normal ranges. Plasma concentrations of 4-TP were within the expected range, whereas plasma/urne with gabapentin plasma levels were below the suggested efficacious concentrations. No adverse effects were detected on clinical and laboratory examinations during and after 4-TP administration. The mare did not get any episode of laminitis in the three months following the treatment.
Grapiprant Uniquely Targets the Prostaglandin EP4 Receptor

PHOSPHOLIPIDS

ARACHIDONIC ACID

PGH₂

PGE₂

PGD₂
PGF₂ₐ

Leukotrienes

Epoxydases

Inhibited by Corticosteroids

Inhibited by NSAIDs (COX 1, 2 Inhibitors)

DP
FP

EP1
EP2
EP3

IP
TP

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