Analgesia and Anaesthesia in animal tests

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Pain

What is pain?

How can I assess it?

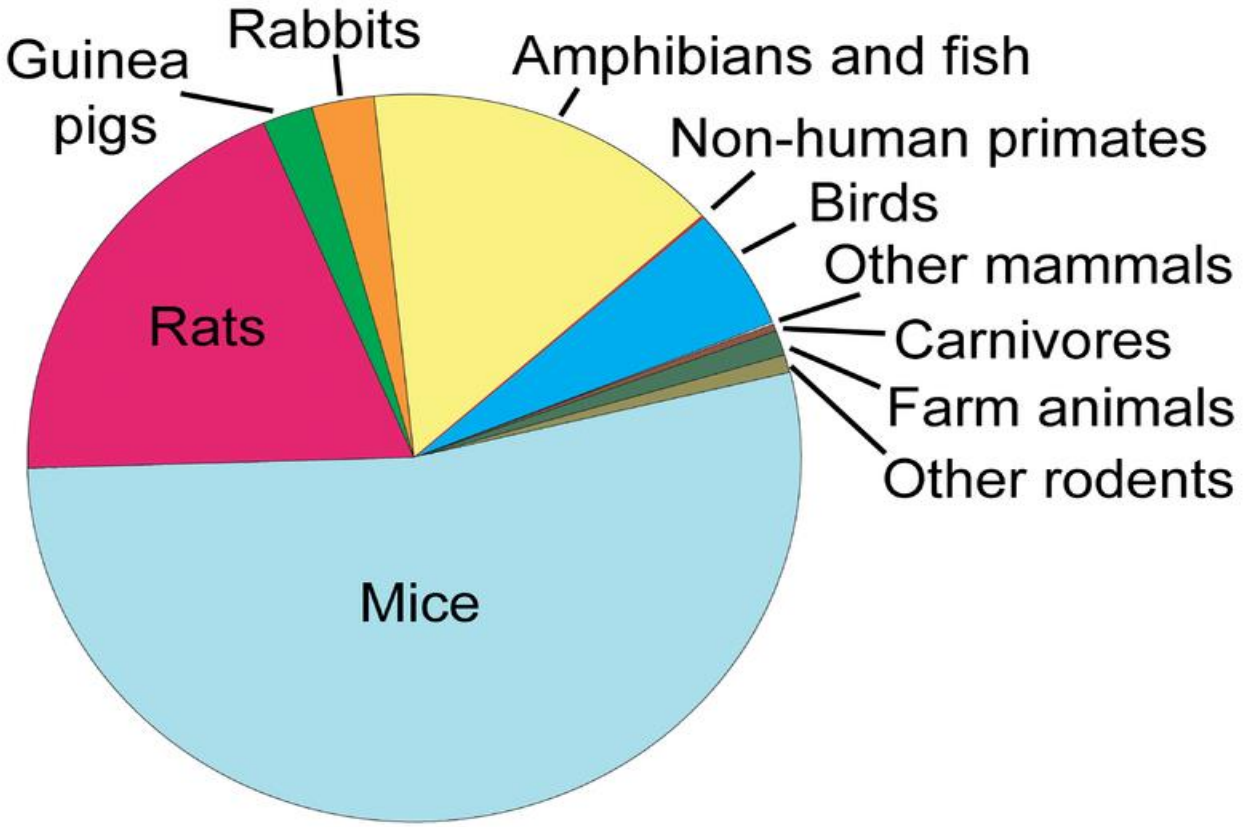
Is a pain test producing the same pain in different animal species?

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?

Research Animals



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

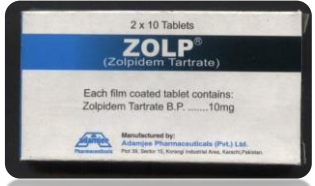
Objective—To evaluate the pharmacokinetics and pharmacodynamics of zolpidem after oral administration of a single dose (0.15 or 0.50 mg/kg) and assess any associated antianxiety and sedative effects in dogs.

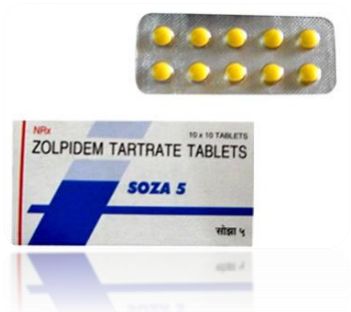
Animals—8 clinically normal sexually intact male dogs of various breeds.

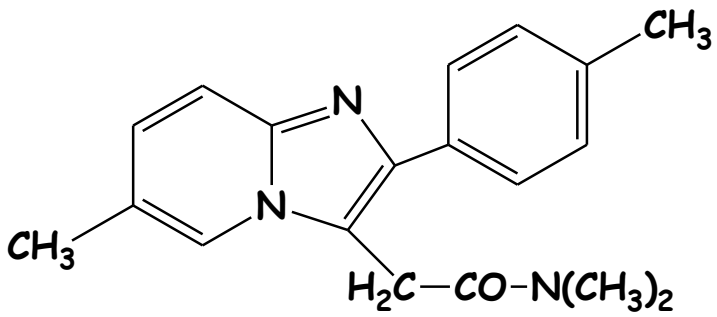
Procedures—Dogs were assigned to 2 groups (4 dogs/group) and administered zolpidem orally once at a dose of 0.15 or 0.50 mg/kg in a crossover study; each dog received the other treatment once after an interval of 1 week. Blood samples were collected before and at intervals during the 24-hour period following 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.

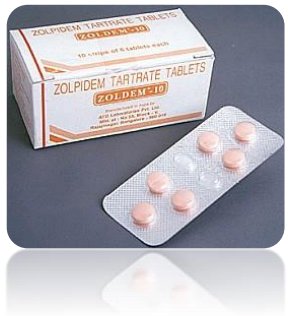
Results—The pharmacokinetic profile of zolpidem in dogs was dose dependent, and the plasma drug concentrations attained were lower than those for humans administered equivalent doses. The lower dose did not result in any clinical or adverse effects, but the higher dose generated paradoxical CNS stimulation 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)









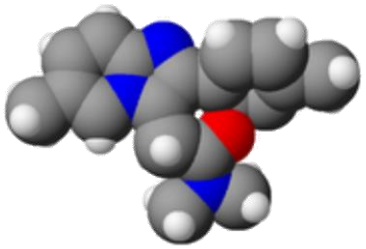
Z-drug class

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 $GABA_A$



ω1

brain

very strong hypnotic properties

ω2

spine

ω3

periferal tissues

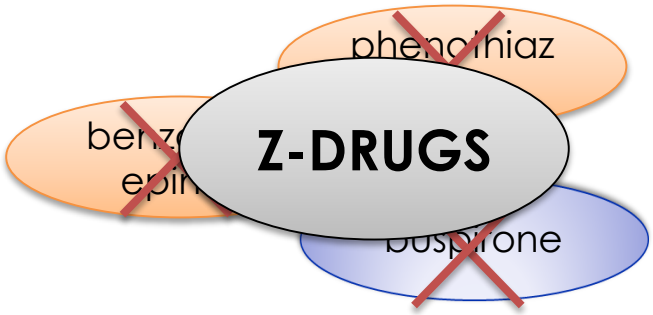
BZDs

very weak anxiolytic, myorelaxant, and anticonvulsant properties

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"

Pain Category	Type of Experiments / Procedures
C	**Experiments / Procedures** **causing little discomfort or stress**
D	**Experiments / Procedures** **causing moderate to severe distress or pain** using anesthesia and / or painkiller
E	**Experiments / Procedures** **causing prolonged or severe clinical distress or pain** without the use of anesthesia and / or painkiller

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; **R**eplacement, **R**eduction, and **R**efinement

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$_2$-agonists

Modulation:

- Can be augmented by injection of local anesthetics or alpha$_2$-adrenergic agonists; gabapentin may also effect modulation

Actions of Analgesics on Pain Processes

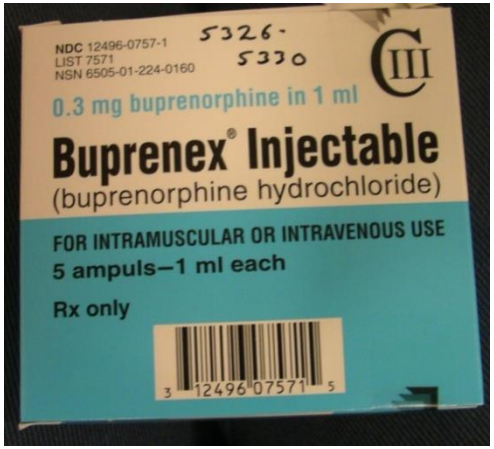
- Pre-emptive analgesia: giving analgesics prior to the noxious stimulus (surgery)
  - By blocking or inhibiting the nociceptive process before it begins, hypersensitivity is prevented
  - May decrease the amount of anesthesia and post-operative analgesia needed
- Multimodal or “balanced” analgesia: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

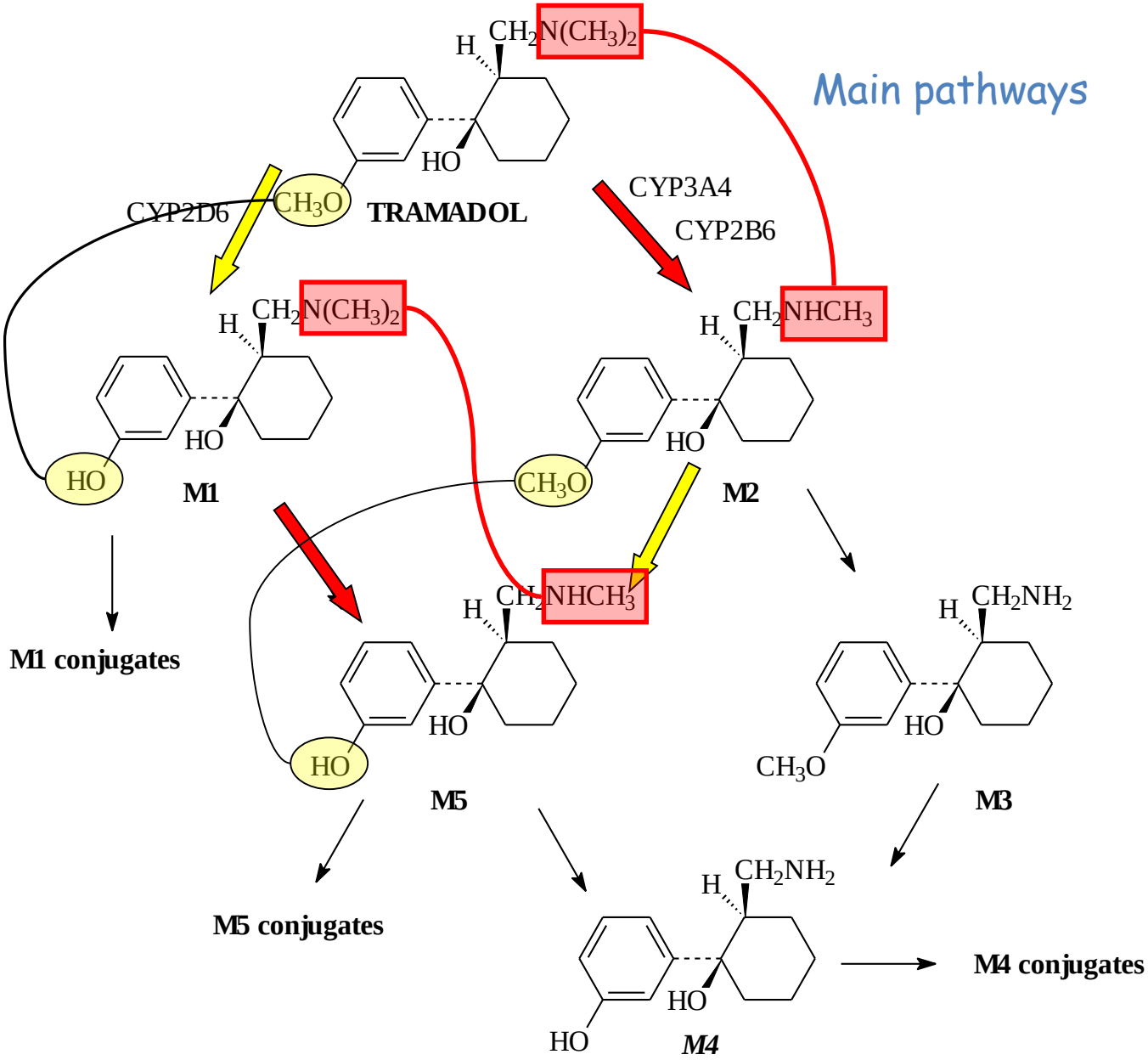
Other classification

Pure (acute)

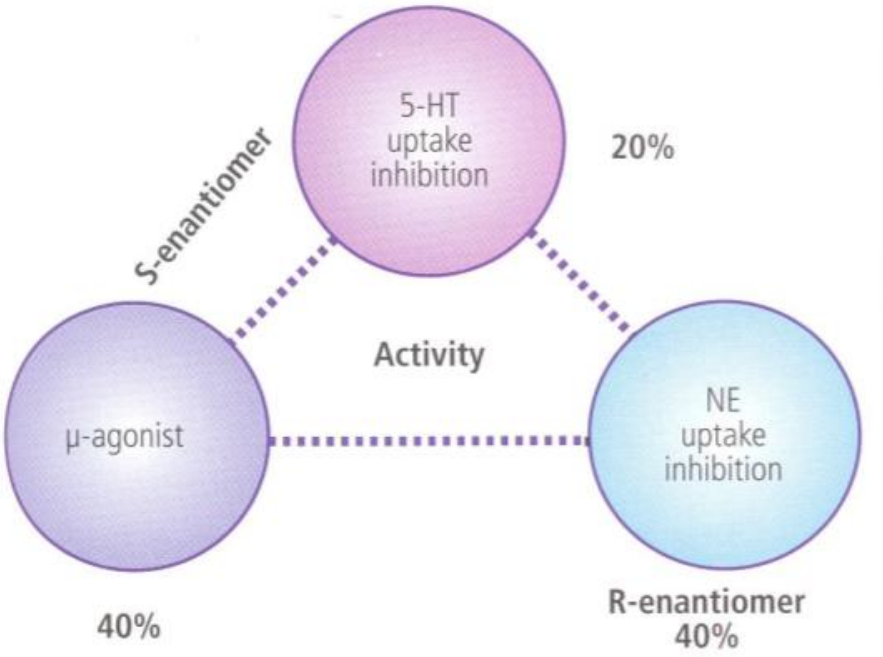
OPIOIDS

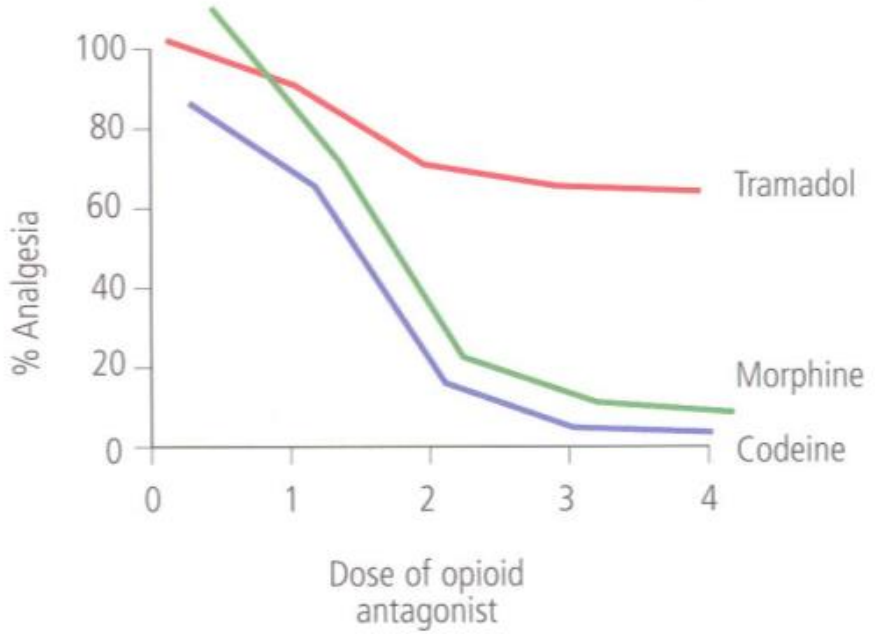
Atipycal (chronic)

Phase I metabolism



Dynamic (summary)





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

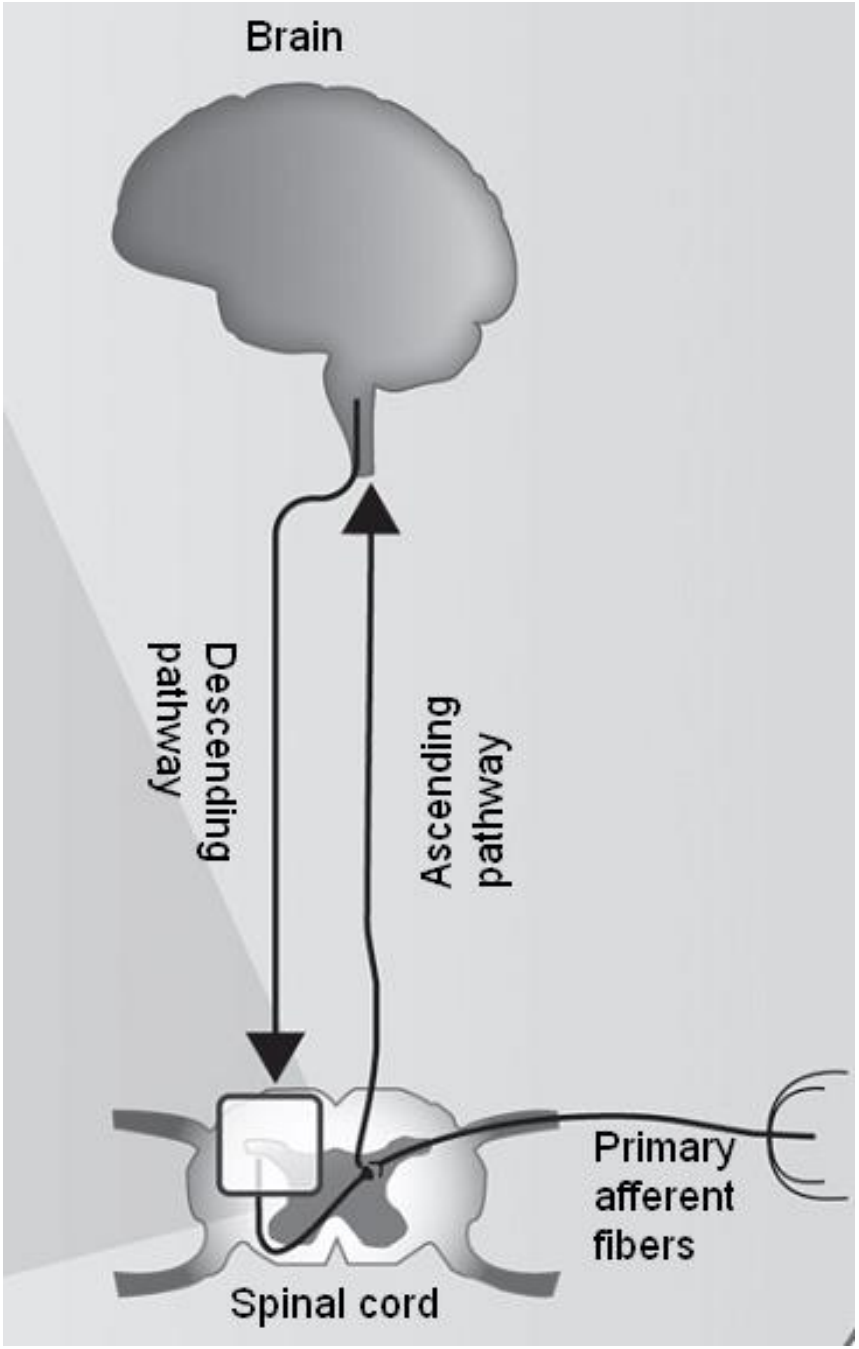
Giorgi et al., 2007, 2010; Shilo et al., 2008

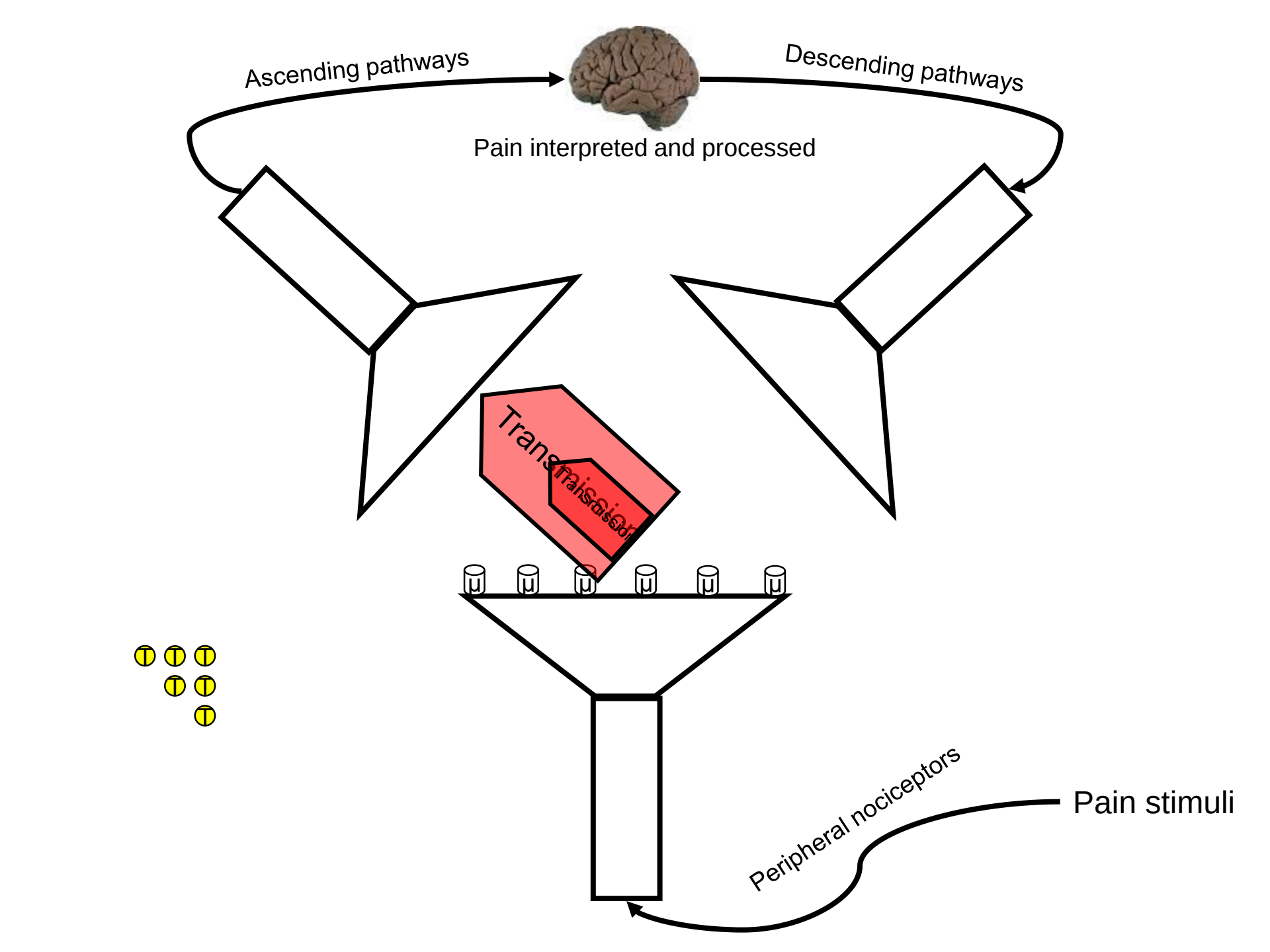
Souza et al., 2009

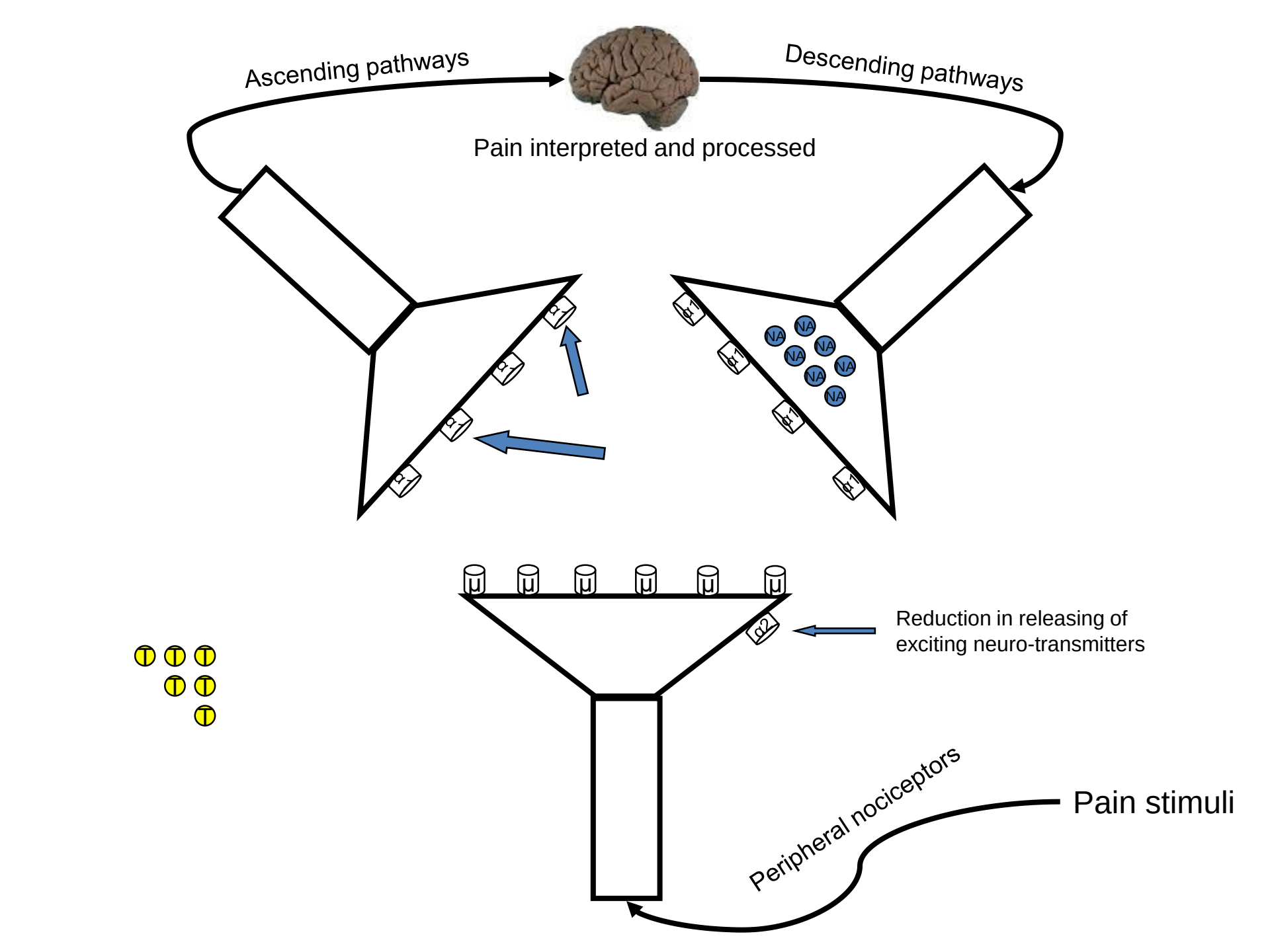
Souza and Cox 2011

Elgazali et al., 2008

Giorgi & Andreoni, 2010


Brain
Descending pathway
Ascending pathway
Primary afferent fibers
Spinal cord


Ascending pathways
Descending pathways
Pain interpreted and processed
Transmission
Transmission
Peripheral nociceptors
Pain stimuli


Ascending pathways
Descending pathways
Pain interpreted and processed
α1
α1
α1
α1
α1
α1
NA
NA
NA
NA
NA
NA
NA
μ
μ
μ
μ
μ
μ
α2
Reduction in releasing of
exciting neuro-transmitters
Peripheral nociceptors
Pain stimuli

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DOI 10.2478/v10181-010-0027-y

Original article

Pharmacokinetics of tramadol and metabolites after injective administrations in dogs

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Vet Res Commun (2009) 33:875–885
DOI 10.1007/s11259-009-9236-1

ORIGINAL ARTICLE

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

New Zealand Veterinary Journal 57(3), 146-152, 2009

Scientific Article

Pharmacokine

Available online at www.sciencedirect.com
ScienceDirect
The Veterinary Journal
www.elsevier.com/locate/tvjl

Veterinary Anaesthesia and Analgesia
Formerly the Journal of Veterinary Anaesthesia

netabolites

M Giorgi*§, S D

Veterinary Anaesthesia and Analgesia, 2014, 41, 297–304 doi:10.1111/vaa.12140

a pilot study

ewska c,

Iranian Journal of Veterinary

RESEARCH PAPER

Effect of o

grapefruit j

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

Giorg

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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

Drug related to arachidonic cascade

NSAIDS

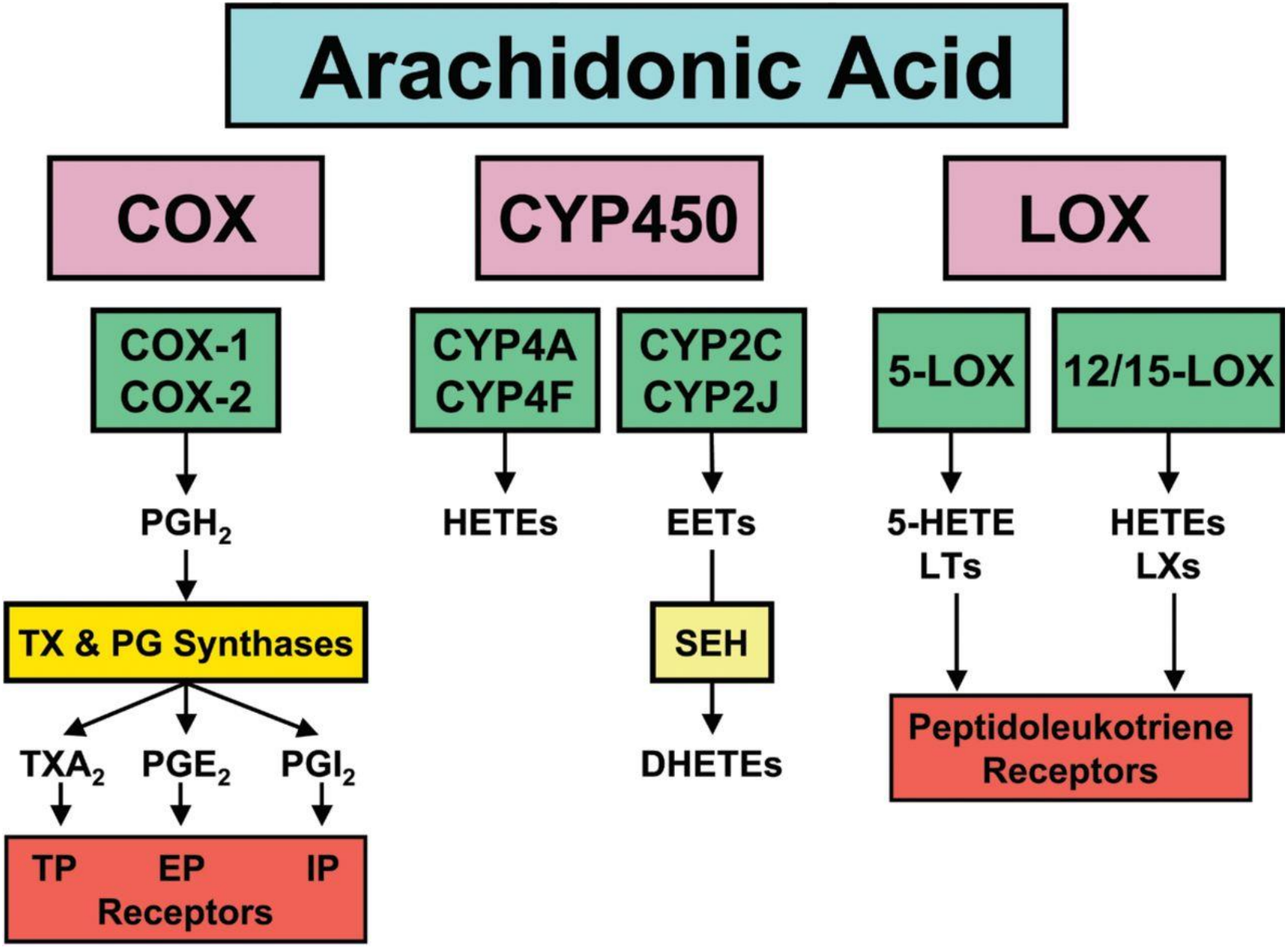
COX 3 (metamizole)
COX ½ (meloxicam)
COX 2 (cimicoxib)

Correlated molecules

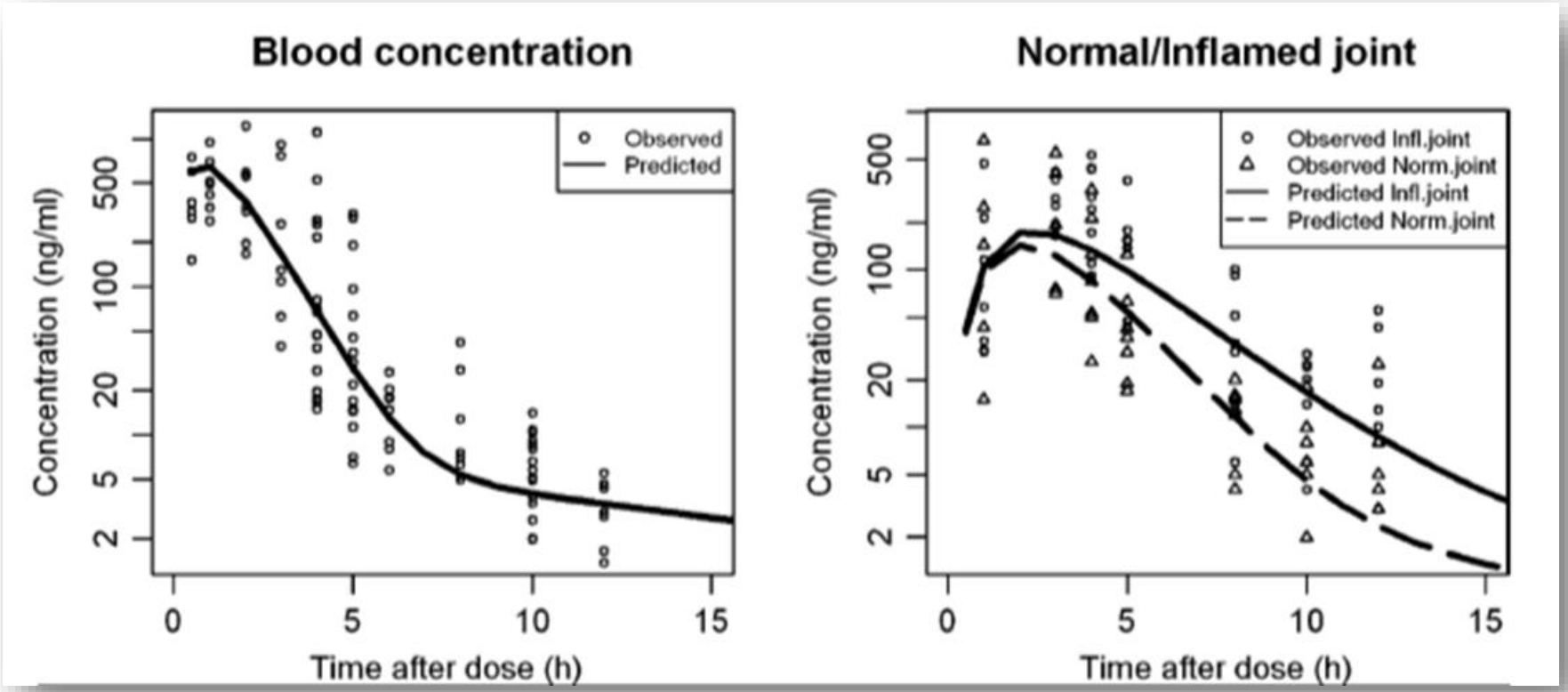
5LP (tepoxalin)

SEHIs (pre clinical study)

EP antagonist (grapiprant)

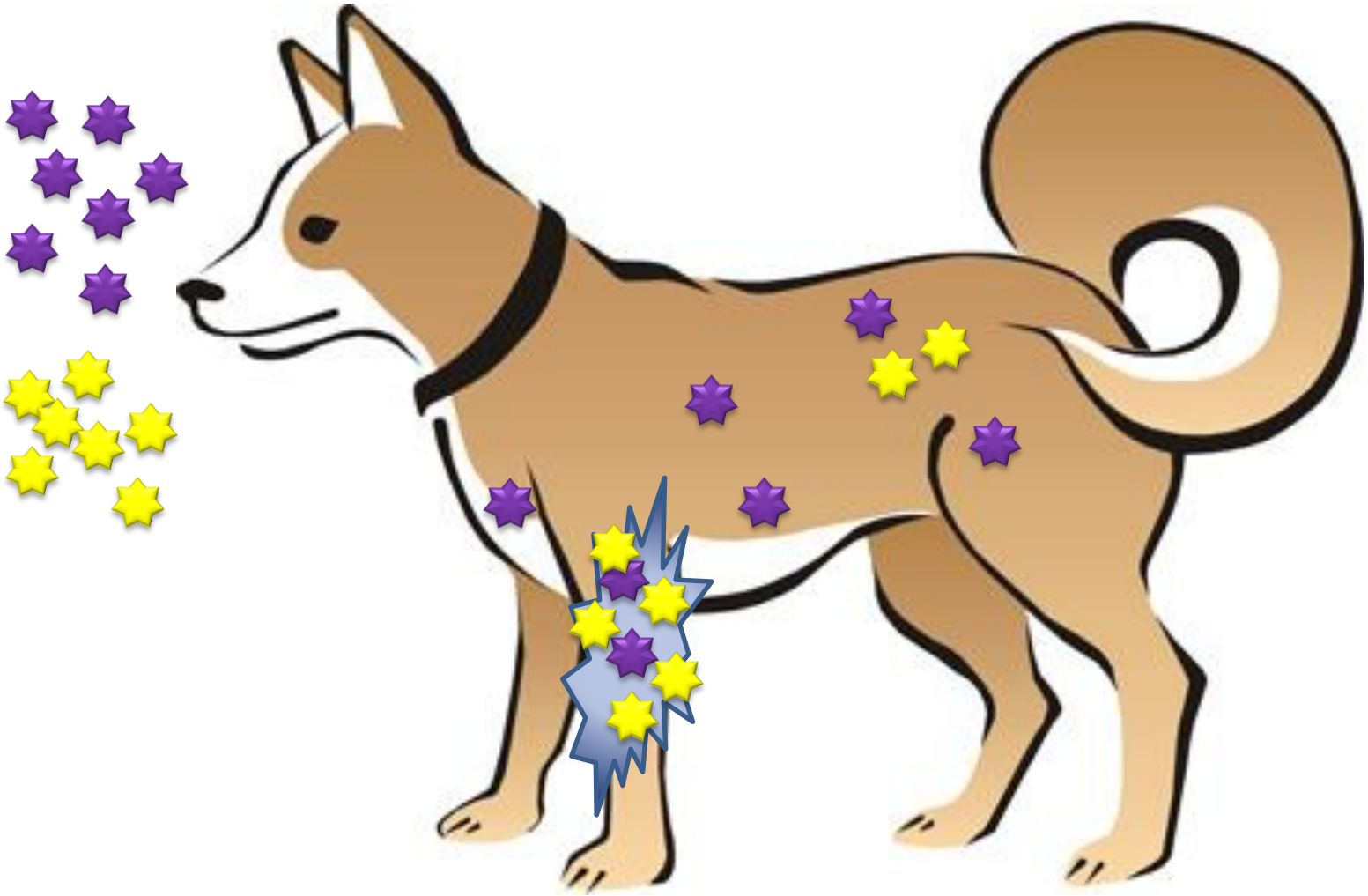

Arachidonic Acid
COX
CYP450
LOX
COX-1
COX-2
CYP4A
CYP4F
CYP2C
CYP2J
5-LOX
12/15-LOX
PGH2
HETEs
EETs
5-HETE
LTs
HETEs
LXs
TX & PG Synthases
SEH
TXA2
PGE2
PGI2
DHETEs
Peptidoleukotriene
Receptors
TP
EP
IP
Receptors

Robenacoxib an example



Silber et al., Pharm Res, 2010

Drug *pKa* and Drug Distribution



Tepoxalin
(Zubrin®- dog)

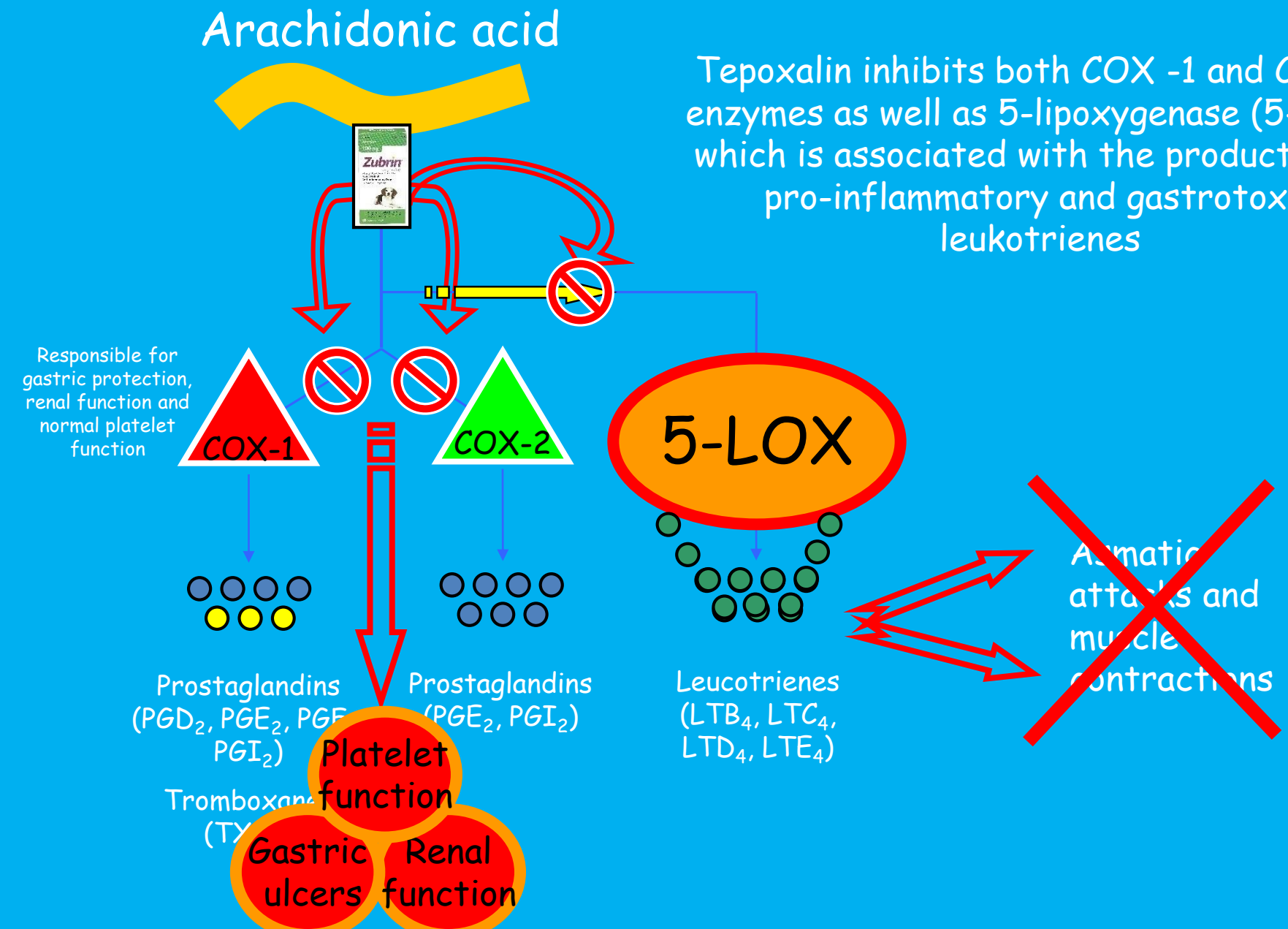
Main effects

Poor GI effects

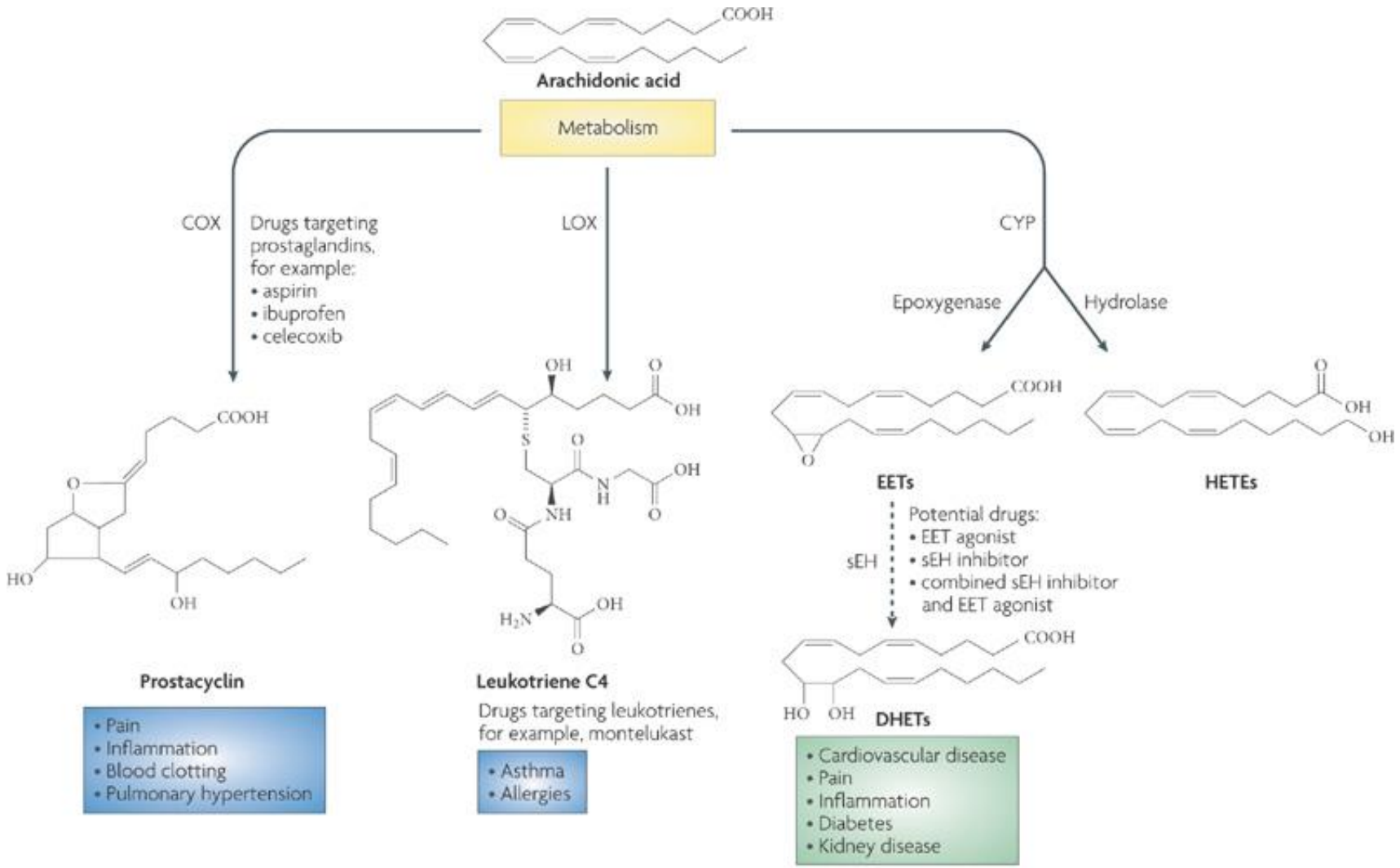
Active metabolite RWJ-20142

Elimination via feces

Pharmacodynamic



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


COOH
Arachidonic acid
Metabolism
COX
Drugs targeting prostaglandins, for example:
• aspirin
• ibuprofen
• celecoxib
LOX
CYP
Epoxygenase
Hydrolase
Prostacyclin
• Pain
• Inflammation
• Blood clotting
• Pulmonary hypertension
Leukotriene C4
Drugs targeting leukotrienes, for example, montelukast
• Asthma
• Allergies
EETs
HETEs
sEH
Potential drugs:
• EET agonist
• sEH inhibitor
• combined sEH inhibitor and EET agonist
DHETs
• Cardiovascular disease
• Pain
• Inflammation
• Diabetes
• Kidney disease

sEHI

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Use of a soluble epoxide hydrolase inhibitor as adjunctive analgesic in a laminitic horse

Alonso G. P. Guedes[1], **Christophe Morisseau**[2], **Albert Sole**[3], **Joao H. N. Soares**[3], **Arzu Ulu**[2], **Hua Dong**[2], and **Bruce D. Hammock**[2]

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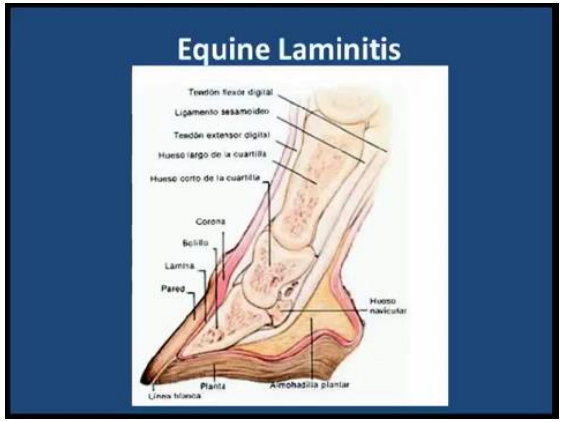
Abstract

A 4-year old, 500 kg Thoroughbred female horse diagnosed with bilateral forelimb laminitis and cellulitis 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 8.5 on day 6, and it increased to 9.5 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, *trans*-4-{4-[3-(4-Trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid (*t*-TUCB), which is an inhibitor of soluble epoxide hydrolase (sEH), to the treatment protocol. Dose 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 equine sEH. Pain scores decreased sharply and remarkably following *t*-TUCB administration and blood pressure progressively decreased to physiologic normal values. Plasma concentrations of t-TUCB, measured daily, were within the expected range, whereas phenylbutazone and gabapentin plasma levels were below the suggested efficacious concentrations. No adverse effects were detected on clinical and laboratory examinations during and after *t*-TUCB administration. The mare did not get any episode of laminitis in the three months following the treatment.

Cat

Horse



Grapiprant Uniquely Targets the Prostaglandin EP4 Receptor