Pain Research: Translational Scientists New Best Friends, and What They Tell Us.

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Pain Research: Translational Scientists New Best Friends, and What They Tell Us.
Translational pain research is not producing new analgesics
Where does translational pain research ‘go wrong’?

**Diagram**

- **Drug Discovery**
  - Pre-discovery: Basic Research and Screening
  - Number of compounds: Tens of thousands
  - Duration: 3-6 years

- **Clinical Trials**
  - Phase I
    - Number of volunteers: 20-100
    - Duration: 6-7 years
  - Phase II
    - Number of volunteers: 100-500
    - Duration: 6-7 years
  - Phase III
    - Number of volunteers: 1,000-5,000
    - Duration: 0.5-2 years

- **FDA Review**
- **Scale-Up to Manufacturing**
- **Phase IV/Ongoing Research and Monitoring**

Preclinical Pain Research

Can We Do Better?

J. David Clark, M.D., Ph.D.

Clinical and pre-clinical pain assessment: Are we measuring the same thing?

C.J. Vierck ++, P.T. Hansson --, R.P. Yezierski

Improve the translation of analgesic drugs to the clinic: animal models of neuropathic pain

N Percie du Sert* and A S C Rice++

Multiple mechanisms have been tested in pain — how can we improve the chances of success?

Ann G Hayes*, Lars Arendt-Nielsen and Simon Tate

Published in final edited form as:

Lost but making progress — Where will new analgesic drugs come from?

David Borsook1, Richard Hargreaves1, Chas Bountra2, and Frank Porreca3,++

Animal models of pain: progress and challenges

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Sensory profiling in animal models of neuropathic pain: a call for back-translation

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Topical review

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Where does preclinical translational pain research ‘go wrong’?

Where **could** preclinical translational pain research **benefit**?

Enhancements to:

- Models (the induced pain state)
- Outcome Measures used in the models
- Relevance of the Target
Models (the induced pain state)

“One can ask whether peripheral nerve injury (e.g. nerve ligation), the acetic acid writhing test, the orofacial injection of formalin, or the intra-articular injection of Freud’s complete adjuvant (FCA) actually mirror any clinical conditions?”

Lascelles & Flecknell 2010. IASP Pain Clinical Updates
Models
Outcome measures

https://www.youtube.com/watch?v=jWqC-5_zXHc
Relevance of target:
Does it play a role in the target disease?
How important is that role?

Goswami et al. 2014

Molecular Signatures of Mouse TRPV1-Lineage Neurons Revealed by RNA-Seq Transcriptome Analysis
A proposition:

Companion animals with naturally-occurring painful disease may enhance the translational pain research paradigm
These naturally occurring painful disease ‘models’ may better reflect the complex genetic, environmental, temporal and physiological influences present in humans.
Verification Bridge: Inform the critical ‘Go / No-Go’ decision making point.
Model fidelity: Osteoarthritis

- Biomechanically, structurally, histologically, genomically, and molecularly human, canine, feline and equine OA are similar.

Outcome Measures:
Measurable dimensions impacted by pain in humans
Gait (joint pain, via limb use)
A Feline-Specific Anti-Nerve Growth Factor Antibody Improves Mobility in Cats with Degenerative Joint Disease–Associated Pain: A Pilot Proof of Concept Study

Improved sleep with NSAID in OA-pain states

Functional linear modeling of activity data shows analgesic-mediated improved sleep in dogs with spontaneous osteoarthritis pain

M. E. Gruen¹, D. R. Samson² & B. D. X. Lascelles³,⁴,⁵

In humans, clear evidence exists that chronic pain interferes with sleep 1. Sleep disturbances decrease quality of life, are associated with higher anxiety and depression2, and worsen chronic pain symptoms3. A common cause of chronic pain is osteoarthritis (OA). Several studies have reported insomnia 4,5, decreased sleep quality 6, and increased self-reporting of pain7 in people with OA. Dogs also suffer from OA that is pathologically and symptomatically similar to humans. These similarities have led to the dog’s emergence as a good naturally-occurring model for understanding human arthritis pain8,9. An improved understanding of the association between OA and sleep in dogs will enhance their use as a model for human OA.

In humans, sleep quality is often measured objectively using actigraphy 7; lower activity counts indicative of less movement are presumed to reflect higher quality sleep. Disturbances of sleep occur due to pain states, but interestingly there are little data on the use of actigraphy to monitor sleep quality in relation to pain relief.

Analgesia-associated modification of sleep in dogs with OA has been previously evaluated by our laboratory using accelerometry and an owner-completed sleep quality questionnaire, the Sleep and Night Time Restlessness Evaluation (SNoRE)10.

The SNoRE is a six-item instrument which asks owners to rate comfort and quality features of their dog’s sleep. In this study, dogs with osteoarthritis wore accelerometers over a five-week period; they received meloxicam (Metacam®, Boehringer-Ingelheim) and placebo, each for two weeks, in a randomized crossover design. Using the SNoRE questionnaire, the study found that dogs receiving meloxicam had improved sleep.
**Function**

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Ability to Rise to Standing From Lying Down</td>
<td>0-10</td>
</tr>
<tr>
<td>8. Ability to Walk</td>
<td>0-10</td>
</tr>
<tr>
<td>9. Ability to Run</td>
<td>0-10</td>
</tr>
<tr>
<td>12. How disabled is your dog by his/her lameness?</td>
<td>Not at all disabled, Slightly disabled, Moderately disabled, Severely disabled, Extremely disabled</td>
</tr>
<tr>
<td>13. How active is your dog?</td>
<td>Extremely active, Very active, Moderately active, Slightly active, Not at all active</td>
</tr>
<tr>
<td>14. What is the effect of cold, damp weather on your dog’s lameness?</td>
<td>No effect, Mild effect, Moderate effect, Severe effect, Extreme effect</td>
</tr>
</tbody>
</table>
Sensory Function: QST Integrity of CPM

Widespread somatosensory sensitivity in naturally occurring canine model of osteoarthritis

David Knazovicky, Erika S. Helgeson, Beth Case, Margaret E. Gruen, William Maixner, B. Duncan X. Lascelles

The effect of spontaneous osteoarthritis on conditioned pain modulation in the canine model

King Wa Chiu, Jon Hash, Rachel Meyers & B. Duncan X. Lascelles
Proof of Concept (POC) Studies

Verification Bridge: Informing the critical ‘Go / No-Go’ decision making point
A randomized clinical efficacy study targeting mPGES1 or EP4 in dogs with spontaneous osteoarthritis

Carol Robertson-Plouch1*, John R. Stille1†, Peng Liu1‡, Claire Smith2§, Dorothy Brown3,4, Margaret Warner1, Leijun Hu1, Matthew J. Fisher1
# Predictability of POC studies in companion animals

<table>
<thead>
<tr>
<th>Drug</th>
<th>Efficacy in Rodents</th>
<th>Efficacy in Dogs</th>
<th>Efficacy in Humans</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSAIDs</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Brown, JVIM, 2013</td>
</tr>
<tr>
<td>Substance P-saporin</td>
<td>Yes</td>
<td>(Yes)</td>
<td>(ongoing)</td>
<td>Brown, <em>Anesthesiology</em>, 2013</td>
</tr>
<tr>
<td>EP4 receptor antagonist</td>
<td>Yes</td>
<td>Yes</td>
<td>(ongoing)</td>
<td>Rausch-Derra, JVIM, 2016;</td>
</tr>
<tr>
<td>Capsaicin (IA)</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
<td>Stevens, <em>Arthritis Rheumatol</em>, 2019; Lascelles, IASP, 2020</td>
</tr>
</tbody>
</table>
The practicalities of running POC studies in companion animals

• Sites and expertise available, allowing veterinary oversight
• Veterinary CRO assistance available
  • GCP; Protocol; Agreements;
• Recruitment (owners; appropriate phenotype)
• Do need sufficient toxicity data in order to test in pet animals
• Ethical Regulations; IACUC; owner consent
• Expense
TRiP

Discovery: face validity & relevance of the target
Discovery

- Companion animals can be pain phenotyped
- Veterinarians have unprecedented access to biological samples
- Interrogate target tissues for target of interest, or in an unbiased way
Uncovering a potential role of artemin/GFRα3 signaling in OA-pain

Ipsilateral and contralateral L4-7 DRG from dogs with unilateral hip OA

No Pain

OA-Pain

Molecular Signatures of Mouse TRPV1- Lineage Neurons Revealed by RNA-Seq Transcriptome Analysis

RT-PCR for a variety of receptors expressed on TRPV1
GFRα3 is upregulated in dogs with naturally occurring OA pain; DRG serving OA-pain joints versus DRG serving normal joints.
Most DRG neurons that are GFRα3 positive are:
• TRPV1 +ve
• TrkA +ve
• RET +ve
• Peripherin +ve
GFRα3 is a GFL growth factor family receptor that complexes with the RET tyrosine kinase to activate intracellular signaling.

**GDNF Family of Ligands (GFL) receptors:**
1. GDNF $\leftrightarrow$ GFRα1
2. NRTN $\leftrightarrow$ GFRα2
3. ARTN $\leftrightarrow$ GFRα3
4. PSPN $\leftrightarrow$ GFRα4

**GDNF family ligands (GFLs):**
1. Glial cell line-derived neurotrophic factor (GDNF)
2. Neurturin (NRTN)
3. Artemin (ARTN)
4. Persephin (PSPN)

Airaksinen & Saarma
Nat Rev Neurosci. 2002
Serum ARTN is elevated in dogs and cats (& humans) with OA-pain compared to healthy controls

- OA dogs and normal dogs (n=26 OA; n=11 normal; p=0.007)

- DJD-pain cats and control cats (n=41 DJD-pain; n=13 control; p=0.011)
Artemin Concentrations in Synovial Fluid Correlate to Limb-use

- Negative values of Symmetry Index (SI) correspond to decreased limb use.
- Increased synovial fluid concentrations of artemin correspond to less limb use (n=8; $R^2=0.62$; $p=0.02$).
Owner-assessed disability shows some relationship to serum ARTN concs (Minnema et al 2020; confirmed with larger ‘n’ in Gupta, submitted)

Total joint pain scores do positively correlate with serum ARTN (Gupta, submitted)
Mouse: 200ng artemin (into paw) produces mechanical, heat and cold sensitivity, in association with decreased limb use
Within a cohort of dogs with OA-pain, serum ARTN does is not associated with remote (from OA joint) sensitivity as measured by QST (n=43)
Early data in mouse model of OA

- ARTN (SC, paw) induces mechanical, heat and cold hypersensitivity
- Increase in GFRα3 in DRG (IHC) with MIA
- No increase in serum ARTN in ‘single joint’ MIA, versus controls; no increase in serum ARTN in bilateral DMM (samples courtesy of A-M Malfiat)
- IA injection of anti-GFRα3 Ab does not improve limb use (MIA model)
- IP injection of anti-ARTN mAb significantly decreases mechanical, heat and cold hypersensitivity in MIA model

N = 8-12 in all groups; replication ongoing
Next steps:

• Detail the expression and co-expression of GFRα3 with TRP channels
• Understand the role of ARTN and GFRα3 in initiating (early) and maintaining (late) OA in different models
  • DMM; MIA
  • Anti-bodies; GFRα3 KO; GFRα3 mutants (non-functional)
• Understand parallel and alternative signaling (GFRα3 vs. GFRα1; RET vs. NCAM)
• Elucidate the downstream targets (TRP receptors)
• Verify findings using ex vivo and in vivo work in pet dogs
Companion animal ‘models’ can contribute to translational pain research in two basic ways

1) **Discovery / Face Validity of Target**: Tissue from naturally occurring disease states may provide vital information about the neurobiology of pain in the natural disease state.

1) **Verification bridge** between rodent preclinical and human clinical studies, testing drugs for efficacy prior to human clinical studies.
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• NIH STTR;
• Private Client Donations;
• Sorrento-ARK;
• Tulane University;
• Virbac;
• Winn Feline Foundation;
• Xalud;
• Zoetis;
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