Impact of Surgery in An Orthotopic Tumor Model: How analgesia can reduce the surgical variable.

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Mice are frequently utilized in Cancer Models

• In the United Kingdom:
  – 12% of all 3.7 million laboratory procedures performed in 2010 were cancer-related
  – More than 420,000 mice and 5,000 rats were used for cancer studies (non-toxicological)

Orthotopic tumor models

• Models of cancer requiring tumor cells be injected into the tissue of origin (orthotopic inoculation)
  – Example: breast cancer cells injected into the mammary gland.

• Benefits:
  – Tumor microenvironment closely approximates aspects of cancer progression, angiogenesis, metastasis and resistance → Greater pre-clinical relevance.

Orthotopic Tumor Inoculation

- Accurate inoculation of cells may require surgery (Workman, 2010; Loi, 2011).
  - Example: laparotomy to visualize liver to inject hepatocellular carcinoma cells

http://flickriver.com/photos/tags/tooble/interesting/
Where there is surgery, analgesia must be considered...

• Unless scientifically justified, post-operative pain must be minimized by providing animals with analgesia (ILAR, 2011).
Our goal was to determine an analgesia regimen for use in a surgical orthotopic cancer model that:

1. would not significantly impact tumor growth
2. minimized the effects of surgery to more closely mimic the spontaneous disease state
Balancing...

- Analgesia
- Surgical Stress
  - Surgery
  - Pain
  - Stress

Accurate Tumor Model
In addressing this balance...

- 3 Major questions:
  - Will providing perioperative analgesia significantly impact the tumor model?
  - Will surgical stress significantly impact the tumor model?
  - Can analgesia reduce the surgical variable resulting in a more accurate tumor model?
Two Studies conducted

• Study 1:
  – Allowed for analgesia and surgery to be evaluated independently and together in an orthotopic tumor model.

• Study 2:
  – Allowed assessment of impact of analgesia on metastatic growth.
Study 1: Design

- Mammary Carcinoma in BALB/c mice
- Single or Multiple dose analgesia
  - Meloxicam, Buprenorphine, Saline
- Laparotomy
Analgesia

- Buprenorphine - 0.2mg/kg SQ
  - Recommended range: 0.05-0.1 mg/kg
- Meloxicam - 10mg/kg SQ
  - Recommended range: 1-2 mg/kg
- Saline (control) – equal volume SQ

- Single (1 pre) or multiple (1 pre and 2 post) dose.

- Both analgesics previously determined to alleviate post-surgical pain in mice.
  - Roughan 2002; Wright-Williams 2007.
Order of Operations...

Anesthesia induction, surgical preparation, SQ injection of analgesia or equivalent volume of saline.  
Injection of either 4T1-luc2 cells or saline into mammary gland.  
Laparotomy or equivalent period of anesthesia.

http://www.bestvetstore.com/category/veterinary-equipment/anesthesia-machine/
http://www.sciencephoto.com/media/156168/enlarge
Bioluminescence Imaging

• Non-invasive, longitudinal method to quantify active tumor growth.
• D-Luciferin reacts with luciferase to produce oxyluciferin, emitting “light” (bioluminescence)
• Intensity of “light” detected is used to approximate the tumor burden.
Measuring post-operative hyperalgesia and allodynia

- Nociceptive measures:
  - Hargreaves: Latency to voluntary withdrawal of paw from a radiant heat source.
  - Electric von Frey: force (g) threshold applied before mouse voluntarily removes paw from mechanical stimuli.
Study 2 Design

• Melanoma in C57BL/6 mice
• Tail vein injection to model metastatic disease
• Single dose analgesia
  – Meloxicam, Buprenorphine, Saline
Question 1: Will providing perioperative analgesia significantly impact the tumor model?
Nonsteroidal Anti-Inflammatories

- Immunocompetent and immunodeficient mice chronically administered NSAIDS demonstrated:
  - Reduced tumor growth
  - Reduced metastasis
  - Increased apoptosis
  - Reduced angiogenesis

Buprenorphine as immunoneutral

- Compared to morphine and fentanyl, which are immunosuppressive, and tramadol, which has immunostimulatory properties, buprenorphine appears to have the fewest effects on the immune system.


Will perioperative Buprenorphine or Meloxicam significantly impact 4T1 or B16 tumor growth?
No significant difference between 4T1 tumor growth (primary tumor model) in single dose analgesia and control groups.
Single dose pre-operative analgesia reduced the post-op secondary hyperalgesia and allodynia measured in the saline treated group.

Hargreaves

Von Frey

- **Saline / Cancer / Surgery**
- **Meloxicam / Cancer / Surgery**
- **Buprenorphine / Cancer / Surgery**
No significant difference between B16 tumor growth (metastatic model) in analgesia and control groups.

**Signal from Thorax**

**Signal from Abdomen**
Result 1

• In the 4T1 mammary carcinoma model and B16 melanoma model, perioperative analgesia **did not** significantly impact primary or metastatic tumor growth (per bioluminescence).
Question 2: Will surgical stress significantly impact the tumor model?
Surgical Stress Increases Tumor Growth

• In humans, rats, and both immunocompetent and immunosuppressed mice laparotomy resulted in:
  – significant decrease in NK cell cytotoxicity
  – significantly greater expression of tumor growth factors
  – significant increase in tumor weight
  – significant increase in tumor seeding of the lungs
  – reduced survival rate compared to non-surgical controls

Pain is immunosuppressive

- Pain stimulates:
  - the HPA axis
  - sympathetic nervous system
- Pain suppresses NK cell activity
- Both result in immunosuppression.

- Consequently, pain promotes tumor development

http://research.unc.edu/ccm/groups/public/@research/@iacuc/documents/content/ccm3_022603.pdf
Did surgical stress significantly impact 4T1 tumor growth?
Within each analgesic group, surgery significantly increased tumor growth compared to non-surgical controls.
Non-Surgery Groups

Surgery Groups

Graph showing total flux (p/s ± 1SEM) over time for different groups:
- Sal/Can
- Mel/Can
- Bup/Can
- Sal/Can/Sur
- Mel/Can/Sur
- Bup/Can/Sur

Time points: 48 hrs, Day 7, Day 12, Day 15, Day 19, Day 22
Result 2

- In the 4T1 model, surgical stress **significantly increased tumor growth** (per bioluminescence).
Question 3: Can analgesia reduce the surgical variable and produce a more balanced model?
Analgesia can reduce impact of surgery on tumor growth

• In acute studies of lung tumor cell retention in rats, both Indomethacin and Buprenorphine:
  – ameliorated the effect of surgery on HPA axis, reducing corticosterone release.
  – preserved NK cell activity
  – maintained tumor growth similar to non-surgery controls.

Page, 2002; Franchi, 2007
Could perioperative analgesia reduce the surgical variable in the 4T1 model?
There was no difference in 4T1 tumor growth in mice treated with multiple doses of peri-inoculation buprenorphine regardless of surgery status.
Surgery significantly increased metastatic growth in the lungs of 4T1 inoculated mice (as measured at time of euthanasia) when no analgesia was provided. However, multiple doses of buprenorphine reduced the surgical effect.
Result 3

- Multiple doses of perioperative buprenorphine reduced the surgical stress variable in the 4T1 mammary carcinoma model.
Conclusions

• While we are not claiming analgesic treatment has no effect on tumors, we did not observe a significant impact of perioperative analgesia on 4T1 or B16 tumor growth as measured by bioluminescence.

• By FAR the larger variable influencing tumor growth and metastasis was surgical stress.

• Further, we demonstrated that analgesia can reduce, not magnify, the surgical variable.
Future Studies

• Sustained release Buprenorphine
• Multi-dose Meloxicam
• Multimodal analgesia (NSAID & Buprenorphine)
• Incorporating immunological markers.
• Meta-analysis of the success or failure of chemotherapeutics developed in orthotopic models requiring and not requiring surgery (with and without provision of perioperative analgesia) in clinical populations.
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http://cutestlife.com/28/awwww-yeah...flowers/
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