Preclinical animal models for adhesion research.

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ESHRE symposium course "System biology tools and preclinical models for translational research in endometriosis and adhesion formation: lessons from cancer and inflammation biology". Leuven, Belgium, September 4-5, 2009
Intraperitoneal Adhesions

Abnormal fibrous connections between surfaces in the abdominal cavity.
Important Clinical Problem

Associated morbidity: intestinal obstruction, chronic pelvic pain, female infertility

Difficulties at the time of reoperation: difficult access, organs injury, bleeding.

Large utilisation of healthcare resources: increased operating, anaesthesia and recovery time, need of blood transfusion, and use of surgical material.

Huge economic impact: e.g. 1.3 billion US$ per year in USA.
Patients without previous surgery

12/115: 10.4%

Patients with previous surgery

198/210: 94.3%

Postoperative adhesions

Adhesion formation

De novo adhesion formation

Adhesion reformation
Pathogenesis

- Normal peritoneum
- Fibrin deposition
- Peritoneal injury

- Fibrin degradation
- Normal healing
- ECM deposition and angiogenesis
- Adhesion formation
Models for adhesion research

1) Consistency, Reliability and Reproducibility
2) Type of animal model: small vs big animals
3) Type of adhesiogenic stimulus
4) Additional variables
5) Statistics and assessment
1. CONSISTENCY, RELIABILITY AND REPRODUCIBILITY

Consistency

 ✓ The consistency of the model must be established before any testing.
 ✓ A surgeon must be thoroughly familiar with the dissection technique. It should be practiced in animals euthanised and then in live animals to ensure that sufficiently extensive and severe adhesions are obtained consistently from one experiment to the next.
 ✓ The method of assessing adhesions must be also well defined before an agent is tested.

Models for adhesion research

1. CONSISTENCY, RELIABILITY AND REPRODUCIBILITY

Reliability

✓ This refers to the ability to rely on the results obtained from a model to make correlations with clinical outcomes, under specific surgical situations.

✓ A model should not be so severe that no agent reduces adhesions (“challenging” model), nor too permissive such that all agents are efficacious (“permissive” models).

Models for adhesion research

1. CONSISTENCY, RELIABILITY AND REPRODUCIBILITY

Reproducibility

✓ This refers to the ability of one investigator to replicate the results of another. The consistency and reliability of the model must be reproduced for each surgeon in a lab. This should also be checked periodically as models may “drift” even with the same surgeon.

✓ Similar precautions must be taken when a model is established in a new lab. If possible a visit to the originating lab and direct observation of every procedure is recommended. Each detail can give variability, variation between laboratories can be considerable, although trends are essentially similar.

### Models for adhesion research

#### 2. TYPE OF ANIMAL MODEL: Small animals

<table>
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<td>- they are being readily available</td>
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(Mouse, rat, rabbit) vs (dog, sheep, pig, horse, monkey)

## Models for adhesion research

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small vs Big animals

**Thickness of the abdominal wall**

✓ Thickness of the entire abdominal wall in mice is equivalent to that of the first muscle layer is in rats.

✓ Same relationship exists between rats and rabbits and between rabbits and dogs.

## Models for adhesion research

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small vs Big animals


Ratio of the peritoneal surface area relative to body weight

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<th>Peritoneal surface area/body weight</th>
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Higher ratio in small animals than in large animals.
## Models for adhesion research

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Higher ratio determines disproportionately higher volume of peritoneal fluid required to coat the entire peritoneal surface.

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small vs Big animals

Transport of molecules from the peritoneum*

✓ The ability of the peritoneum to transport molecules is similar in all mammals
✓ Highly dependent on the surface area
✓ Higher in small animals, thus elimination of molecules will be faster in smaller animals.

Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small animals

✓ Mice and rats, in contrast with rabbits, do not require sterile conditions for surgery.
Models for adhesion research

2. TYPE OF ANIMAL MODEL:

**Mice: Advantages**

✅ One of the best-developed animal models
✅ Availability of inbred animals
✅ Availability of genetically manipulated animals, e.g. knockout mice, mice with under or over expression of specific genes.
✅ Animals with altered immune system, e.g. *nude* and SCID mice (human cells in mice).
✅ Availability of many specific assays and monoclonal antibodies
✅ Drug screening
Inbred Animal Models: Definition

- Inbred strains are animals which are nearly identical to each other in genotype due to long inbreeding. Mating of brother-sister pairs for 20 generations will result in lines that are roughly 98% genetically identical, usually sufficient to be considered an inbred strain (compare to identical twins or clones which are 100% genetically identical, or fraternal twins or normal siblings, which are roughly 50% identical).
Models for adhesion research

2. TYPE OF ANIMAL MODEL:

Inbred Animal Models: *Advantages*

- Highly consistent
- Essentially genetically identical - isogenicity
- Highly reproducible across individuals and generations
- Test different chemicals and doses on essentially the same genotype
- Minimize phenotypic variances
- Use multiple strains to ensure that one of the strains is sensitive to a given toxicant
Inbred Animal Models: Limitations*

• Using a single strain of inbred mice cannot reflect the natural variation of the human patient population.
• Indeed, marked strain differences exist in the susceptibility of mice to atherosclerosis, autoimmune diseases, stroke, asthma and adhesion formation (Molinas 2005).
• <10% of new drugs tested in clinical trials receive Food and Drug Administration (FDA) approval.
• Example “A study of drug efficacy using a disease model in a single inbred mouse strain could be compared with a clinical drug trial performed in an isolated South Pacific island population”.

Models for adhesion research

2. TYPE OF ANIMAL MODEL:

- Different animal models:
  - rat (~200)
  - rabbit (~140)
  - mouse/pig/dog (~30)
  - horse (4: model?)
  - sheep/monkey (2: model?)

www.pubmed.org
Models for adhesion formation

3. TYPE OF ADHESIOGENIC STIMULUS

Abrasion, crushing, desiccation, incision, excision, electrocautery, laser injury, thermal injury, chemical injury, radiation injury, foreign body-tissue irritation *.

Models for adhesion formation

4. ADDITIONAL VARIABLES

Bleeding, ischaemia, contamination/infection, anastomosis, other pathology (endometriosis, cancer), formation vs reformation, laparoscopy vs laparotomy. *

Models for adhesion formation

5. STATISTICS AND ASSESSMENT

- Study size: it will depend on whether data are used for screening or definitive purposes.
- Screening studies: several candidates, further studies; smaller sample size (n=3-8), higher p value (e.g. 0.1-0.2) and lower power (e.g. 60%).
- Definitive studies: end of one research phase; number of animals should provide sufficient power (e.g. 80%).

Models for adhesion formation

5. STATISTICS AND ASSESSMENT

Block Randomization

- If our experiment has 6 groups and 8 animal/group:
- NO: Day 1: 8x group 1; Day 2: 8x group 2, etc.
- YES: Day 1: group 1,3,6,5,4,2
  Day 2: group 2,5,1,4,3,6.... Etc

- To avoid day to day variability, learning curve, climatic conditions, surgeon fatigue
Models for adhesion formation

The laparoscopic mouse model

- Easier to handle
- Cheaper
- Available quickly
- Inbred mice
- Knock out mice
- Not need of steril conditions
- m Antibodies
The laparoscopic mouse model

Previous work of:
Drs Yesildaglar, Ordonez, Molinas, Elkilani, Mynbaev, Binda, Schonman

Actual work of: Drs Corona, Verguts

Supervision of Professor Philippe Koninckx
The laparoscopic mouse model
Set up

- Insufflator
- Ventilator
- Humidifier
- Insufflation pressure
- Water valve
- CO₂
- Endotracheal intubation
- Endoscope
- Trocar
- Needle

Chamber at 37°C
The laparoscopic mouse model

Set up
The laparoscopic mouse model

Induction of adhesions

Standardised bipolar lesions in uterine horns and pelvic sidewalls during laparoscopy
The laparoscopic mouse model

Scoring of adhesions

After 7 days, blindly, under microscopic vision, during laparotomy

<table>
<thead>
<tr>
<th>Quantitative scoring</th>
<th>Qualitative scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesions (%) = ( \frac{\sum \text{length of the individual attachments}}{\text{Length of the lesion}} )</td>
<td>Extent: 0 - 4</td>
</tr>
<tr>
<td></td>
<td>Type: 0 - 3</td>
</tr>
<tr>
<td></td>
<td>Tenacity: 0 - 3</td>
</tr>
<tr>
<td></td>
<td>Total: 0 - 10</td>
</tr>
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The formula for Adhesions (%) is given as:

\[
\text{Adhesions} = \frac{\sum \text{length of the individual attachments}}{\text{Length of the lesion}}
\]
The laparoscopic mouse model

SOME OF OUR RESULTS
CO₂ pneumoperitoneum is a cofactor in adhesion formation

- Adhesions increase with duration of surgery
- Adhesions increase with insufflation pressure
- Adhesions are similar with CO₂ and Helium pneumoperitoneum
- Adhesions decrease after addition of oxygen

**CO₂ pneumoperitoneum is a cofactor in adhesion formation**

- Adhesions increase with the addition of more than 3% Oxygen to the pneumoperitoneum (Elkelani OA, Binda MM *et al*., *Fertil Steril* 2004)

- Hypercarbia/Acidosis (Molinas *et al*, *Fertil Steril* 2004)

- Manipulation during pneumoperitoneum increases adhesions (Schonman R *et al*, *J Minim Invasive Gynecol*. 2009)
CO\textsubscript{2} pneumoperitoneum is a cofactor in adhesion formation

**Hypothesis Mesothelial Hypoxia**

✓ Consistent with the absence of pneumoperitoneum-enhanced adhesion in mice knockout for HIFs, VEGF-A, VEGF-B, PlGF and PAI-1.

*Molinas et al, Fertil Steril 2003*
Genetic background has an influence in adhesion formation

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>Strain</th>
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<tbody>
<tr>
<td>Outbred</td>
<td>Swiss</td>
</tr>
<tr>
<td>Outbred</td>
<td>NMRI</td>
</tr>
<tr>
<td>Inbred</td>
<td>BALB/c</td>
</tr>
<tr>
<td>Inbred</td>
<td>C57BL/6J</td>
</tr>
<tr>
<td>Inbred</td>
<td>FVB</td>
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Molinas CR, Binda MM et al, Fertil Steril, 2005
Hypothermia reduces adhesion formation

Experimental design: 60 min CO₂ PP, humidified gas for PP, little flow = No desiccation, Chamber at 37°C, modulate mouse body temperature: around 37°C: ventilation with humidified gas, around 36°C: ventilation with non-humidified gas, around 32°C: placing mouse in/outside the 37°C chamber

Two way ANOVA

a p<0.01 vs group I
b p<0.0001 vs group II


Experimental design: 60 min CO₂ PP, humidified gas for PP, little flow = No desiccation, Chamber at 37°C, modulate mouse body temperature: around 37°C: ventilation with humidified gas, around 36°C: ventilation with non-humidified gas, around 32°C: placing mouse in/outside the 37°C chamber

P=0.02, Pearson
Hypothermia reduces adhesion formation

Figure 4. Relationship between body temperature and adhesion formation. Individual values of the mean of body temperature between $T_{20}$ and $T_{80}$ with their respective proportion of adhesions are depicted for pneumoperitoneum-enhanced adhesion for experiments I and III. $P = 0.004$ (Pearson correlation).

Desiccation increases adhesion formation

Wilcoxon

\[ a \ p<0.05 \text{ vs group I} \]
\[ b \ p<0.05 \text{ vs group III} \]

Desiccation increases adhesion formation

Wilcoxon

a p<0.05 vs group I
b p<0.05 vs group III

Hypothermia reduces adhesion formation


Wilcoxon

\(^a\) p<0.05 vs group I

\(^b\) p<0.05 vs group III
Adhesion Prevention in our laparoscopic mouse model

Lesion+60 min PP+Desiccation+no training
Adhesion Prevention in our laparoscopic mouse model

Lesion + 60 min PP + Desiccation + no training

Lesion + 60 min PP + Desiccation + training
Adhesion Prevention in our laparoscopic mouse model

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Adhesion Prevention in our laparoscopic mouse model

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Lesion+60 min PP+3%O₂+Low temperature = Conditioning the PP
Adhesion Prevention in our laparoscopic mouse model

Lesion + 60 min PP + Desiccation + no training

Lesion + 60 min PP + Desiccation + training

Lesion + 60 min PP

Lesion + 60 min PP + 3% O₂ + Low temperature = Conditioning the PP + Ca²⁺ channel blocker, Phosph + Dexamethasone + Barriers

Summary of our results in the laparoscopic mouse model:

Adhesion formation is influenced by:

- Genetic background
- Duration and pressure of PP
- Type of gas and its humidification
- Body temperature: hypothermia reduces AF
- Manipulation: good surgeon training is very important
Summary of our results in the laparoscopic mouse model:

✓ Best way to reduce adhesion: conditioning the PP (humidified CO$_2$ + 3% O$_2$ and low temperature) + combination of products + good surgeon training.

Conclusions: Animal models for adhesion formation

- Small animal models, i.e. mouse, rat and rabbit are the most used models for screening experiments (mouse and rat: inbred strains, no need of sterility during surgery)
  - Good price, easy to handle, available quickly

- Before starting any study, the consistency, reliability and reproducibility of the animal model should be checked.
Many Thanks

City Hall, Leuven, Belgium