The animal intensive care unit – how to design animal experiments in shock research

Peter Radermacher, Pierre Asfar
Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?
"...Longer experiments with more advanced supportive care would allow better mimicry of the later stages of sepsis and multiorgan failure, permitting the testing of drugs in a more realistic setting...."

"...For example, options to enrich the pre-clinical portfolio include the study of animals that are more genetically diverse, are older, or have preexisting disease...."

**Marini et al:** Critical care evidence – new directions. 
**JAMA 2015;313:893**

"...Development of appropriate experimental animal models or other preclinical research models should be considered a high priority of future research agendas...."
“...Would you as a critical care physician accept data on a septic patient who was not resuscitated? Would you accept data from a drug study on an intensive care patient who was not only not resuscitated with fluid but who did not even have blood pressures and heart rates monitored?... If the animals are resuscitated, is the resuscitation to a specific physiologic variable?... The pathophysiology and outcome of an unresuscitated, unmonitored, septic patient is certainly different....”
Survival (%)

Time (h)

Control group

Do we need the „Animal ICU“?

Yes!

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?
Do we need the „Animal ICU“?

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mice as models for ALI/ARDS/shock

**Pro:**
- Genetically-modified strains available
- Homogeneous groups:
  - Incest strains -> little genetical variability
  - Gender selection possible
  - Young, healthy *or* specific co-morbidity, age
- Easy control of body temperature
- Numerous test systems available

**Con:**
- Size -> small blood volume, difficult instrumentation
- „non-shivering“ thermogenesis
- different mediator release/response
Genomic responses in mouse models poorly mimic human inflammatory diseases


Genomic responses in mouse models greatly mimic human inflammatory diseases

Keizo Takao and Tsuyoshi Miyakawa

ABANDON THE MOUSE RESEARCH SHIP? NOT JUST YET!


PNAS 2013;110:3507

PNAS 2015;112:1167

Shock 2014;41:463
LPS doses

**Human volunteers:**
- 4 ng/kg bolus (Preas et al, AJRCCM 2001; 164:620)
- 2 ng/kg bolus (Michaeli et al, Clin Nutr 2007;26:70)

**Mice, endotoxin-induced acute lung injury:**
- 0.4 mg/kg bolus nasal (Dreymüller et al, EMBO Mol Med 2012;4:412)
- 1.5 mg/kg bolus i.v. (Mangalmurti et al, Blood 2009;113:1158)
- 4 mg/kg bolus intratracheal (Mekontso-Dessap et al, AJRCBM 2012;46:541)

**Pigs, endotoxin-induced „septic“ shock:**
- 10-40 ng/kgxmin infusion (Hauser et al, CCM 2005;33:2034)
Human septic shock:
• 77 ± 10 µM (controls: 29 ± 4; non-septic trauma 13 ± 2 µM) (Ochoa et al, Ann Surg 1991;32:13)
• 47 (28-77) µM (Watson et al, CCM 2004;32:13)

Mice, CLP-induced septic acute lung injury:
• 102 (47-121) µM (Barth et al, CCM 2006;34:307)
• 161 ± 23 µM (Wang et al, Am J Pathol 2012;180:505)

Pigs, fecal peritonitis-induced septic shock:
• 17 (11-30) µM (before shock: 21 (12-48) µM) (Simon et al, Crit Care 2009;13:R113)
**Anatomical and Physiological Similarities of the Kidney**

<table>
<thead>
<tr>
<th>Mice, rats and rabbits</th>
<th>Pig, monkeys and HUMANS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Unilobular, unipapillar kidney</td>
<td>• Multilobular, multipapillar kidney</td>
</tr>
<tr>
<td>• Urine empties directly into renal pelvis (no branched calicel network)</td>
<td>• Urine empties into calicel network into renal pelvis</td>
</tr>
<tr>
<td>• Segmental arteries are bypassed</td>
<td>• Interlobular and segmental arteries</td>
</tr>
</tbody>
</table>

Simmons, J Urol 2008;180:19
The „Mouse Intensive Care Unit“-concept
Anesthetics
Noradrenaline
Colloids
$^{13}$C$_6$-glucose-isotope

Inspiratory/expiratory CO$_2$/O$_2$: calorimetry
$^{13}$CO$_2$-Infrared-Spectrometry

Tracheostomy

LV pressure-volume loops

Catheter A. carotis

Temperature probe

Closed-loop body temperature control

Remission-spectrophotometry
Laser-Doppler-Flowmetry

Liver

A. mes. sup.

Bladder catheter

V. portae

Ultrasound-Doppler flowmetry

Catheter V. jugularis

Arterial pressure, heart rate

Urine output, Creatinine clearance

Catheter A. femoralis

V. portae
Mice, 6 hrs; FiO₂ 0.5, RR 180/min, Vt 8 mL/kg, PEEP 6 cmH₂O, Recruitment 30 cmH₂O/1 sec every 5 / 60 min vs. no recruitment
## Cardiopulmonary, Histologic, and Inflammatory Effects of Intravenous Na$_2$S After Blunt Chest Trauma-Induced Lung Contusion in Mice

**Wagner et al:**

<table>
<thead>
<tr>
<th></th>
<th>Hert rate (beats/min)</th>
<th>MAP (mmHg)</th>
<th>pH</th>
<th>Cpl (µL/cmH$_2$O)</th>
<th>PaO$_2$/FiO$_2$ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start</strong></td>
<td><strong>325</strong> (311;350)</td>
<td><strong>60</strong> (58;71)</td>
<td><strong>7.31</strong> (7.27;7.33)</td>
<td><strong>64</strong> (60;71)</td>
<td><strong>362</strong> (314;510)</td>
</tr>
<tr>
<td><strong>End</strong></td>
<td><strong>370</strong> (340;441)</td>
<td><strong>72</strong> (66;78)</td>
<td><strong>7.38</strong> (7.35;7.42)</td>
<td><strong>70</strong> (64;81)</td>
<td><strong>564</strong> (456;614)</td>
</tr>
</tbody>
</table>

**Total lung injury score** ($\Sigma$ dystelectasis/atelectasis + edema/hemorrhage + leukocyte infiltration + thickened alveolar membrane)

- **Sham** 1.5 (1.5,2.25)
- **TxT** 4.5 (3.0,5.0)
The „Pig Intensive Care Unit“-Concept
Ventral view of the abdominal viscera of the pig in situ.
Measurements:

- Modified Glasgow Coma Scale
- Response to painful stimuli
- Microdialysis for glutamate, pyruvate, lactate and glucose
- bilateral measurement of ICP (CPP), $p_t O_2$ and temperature
- Mitochondrial respiration and ROS production in PBMCs and neutrophils
- Electron spin resonance

Post-mortem:

- Nissl-Staining
- Western blots
- Immunohistochemistry
- High resolution respirometry
George E.P. Box, FRS (1919-2013): 'All models are wrong, but some are useful'
**Tucker A, et al:**
Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude.
*Am J Physiol 1975;228:762*

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>Altitude (4500m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sheep</strong> 42 days</td>
<td>20 ± 1</td>
<td>23 ± 2</td>
</tr>
<tr>
<td><strong>Dog</strong> 42 days</td>
<td>26 ± 2</td>
<td>28 ± 1</td>
</tr>
<tr>
<td><strong>Pig</strong> 42 days</td>
<td>27 ± 1</td>
<td>72 ± 7</td>
</tr>
</tbody>
</table>
Kuriyama, et al:
Role of collateral ventilation in ventilation-perfusion imbalance.
*J Appl Physiol* 1984;56:1500

Dogs, pigs; isolated lung region FiO₂ 13 %, remainder 30 %: Presence or not of alveolar collateral ventilation
Do we need the „Animal ICU“?

Large vs. small animal models?

Both!

Pre-existing co-morbidity?

Translation to the clinics?
Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?
Su et al:
Epidemiology of sepsis in Germany: results from a national prospective multicenter study.
*Intensive Care Med* 2007;33:606-18

ICU length of stay 12 - 19 days

The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation

<table>
<thead>
<tr>
<th>Hospital</th>
<th>ICU</th>
<th>Mechanical Ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 (19-64)</td>
<td>22 (13-36)</td>
<td>17 (10-29)</td>
</tr>
</tbody>
</table>
### Recurrent Recruitment Manoeuvres Improve Lung Mechanics and Minimize Lung Injury during Mechanical Ventilation of Healthy Mice

Lucy Kathleen Reiss*, Anke Kowallik, Stefan Uhlig

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Monitored parameters</th>
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<tbody>
<tr>
<td></td>
<td>Lung functions</td>
</tr>
<tr>
<td>Ventilation</td>
<td>Vₜ [ml/kg]</td>
</tr>
<tr>
<td>30 min</td>
<td>7/10</td>
</tr>
<tr>
<td>60 min</td>
<td>8</td>
</tr>
<tr>
<td>140 min</td>
<td>30/10</td>
</tr>
<tr>
<td>150 min</td>
<td>8</td>
</tr>
<tr>
<td>4 h</td>
<td>25/7</td>
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<tr>
<td>4 h</td>
<td>20/6</td>
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<tr>
<td>4 h</td>
<td>30</td>
</tr>
<tr>
<td>4 h</td>
<td>20/7</td>
</tr>
<tr>
<td>4 h</td>
<td>8</td>
</tr>
<tr>
<td>5 h</td>
<td>30/6</td>
</tr>
<tr>
<td>5 h</td>
<td>15/7.5</td>
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<tr>
<td>6 h</td>
<td>24</td>
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<tr>
<td>6 h</td>
<td>12</td>
</tr>
<tr>
<td>6 h</td>
<td>20/10</td>
</tr>
<tr>
<td>4 h/8</td>
<td>2</td>
</tr>
<tr>
<td>8 h</td>
<td>12</td>
</tr>
<tr>
<td>6 h</td>
<td>16/8</td>
</tr>
</tbody>
</table>

**S zabari et al:**
Relation between respiratory mechanics, inflammation, and survival in experimental mechanical ventilation.

*AJRCMB 2018 doi: 10.1165/rcmb.2018-0100OC*


Vuda et al: Effects of catecholamines on hepatic and skeletal muscle mitochondrial respiration after prolonged exposure to faecal peritonitis in pigs. *Innate Immun* 2011;1-14


22h

24h

24h

27h

48h

72h

96h

104h
Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

As long as possible!??!

Translation to the clinics?
Do we need the „Animal ICU“?
Large vs. small animal models?
How long should animal models last?
Pre-existing co-morbidity?
Translation to the clinics?
Severe Sepsis and Septic Shock

Derek C. Angus, M.D., M.P.H., and Tom van der Poll, M.D., Ph.D.


“...For example, options to enrich the pre-clinical portfolio include the study of animals that are more genetically diverse, are older, or have preexisting disease. ...“
Woran wir sterben


- Stroke $5.8 \cdot 10^6$ (24 %)
- MI $7.0 \cdot 10^6$ (7 %)
- COPD $3.8 \cdot 10^6$ (2.5 %)
- Diabetes $1.3 \cdot 10^6$
Roth Händle
4 days / week over 3-4 weeks
Hartmann et al:
In-depth characterization of the effects of cigarette smoke exposure on the acute trauma response and haemorrhage in mice.
*Shock, doi: 10.1097/SHK.0000000000001115*

\[ p=0.037 \]
Human-like atherosclerosis in minipigs: a new model for detection and treatment of vulnerable plaques

Troels Thim
**Hartmann et al:** Effects of hyperoxia during resuscitation from hemorrhagic shock in swine with pre-existing coronary artery disease. *Crit Care Med 2017;45:e1270-9*

**Pigs with pre-existing atherosclerosis:** FiO2 1.0 vs. standard treatment during the first 24 h (of 48 h) after 3-h hemorrhage

- **LDL**
  - Low fat diet: 5 mmol/L
  - High fat diet: 15 mmol/L
  - *p* < 0.001

- **MAP baseline**
  - Low fat diet: 100 mmHg
  - High fat diet: 120 mmHg

- **Plasma creatinine**
  - Low fat diet: 60 µmol/L
  - High fat diet: 140 µmol/L
  - *p* < 0.001

- **Tau**
  - Low fat diet: 10 ms
  - High fat diet: 20 ms
  - *p* = 0.02

- **Norepinephrine**
  - Normoxia: 2 µg/kg*min*
  - Hyperoxia: 4 µg/kg*min*
  - *p* = 0.02

- **LDL**
  - Low fat diet: 5 mmol/L
  - High fat diet: 20 mmol/L
  - *p* < 0.001
Do we need the „Animal ICU“?
Large vs. small animal models?
How long should animal models last?
Pre-existing co-morbidity?

Must be included!
Do we need the „Animal ICU“?
Large vs. small animal models?
How long should animal models last?
Pre-existing co-morbidity?
Translation to the clinics?
In summary, \textit{L-NMMA} and \textit{NOR} were equally effective in maintaining MAP during long-term hyperdynamic porcine endotoxic shock.

\textit{López et al.}


\textit{CCM 2004;32:21}

\textit{Conclusions:} In this study, the nonselective nitric oxide synthase inhibitor 546C88 increased mortality in patients with septic shock.
Simon et al.
Comparison of cardiac, hepatic and renal effects of arginine vasopressin and noradrenaline during porcine fecal peritonitis: a randomized, controlled trial. *Crit Care* 2009;13:R113

Low-dose AVP appears to be safe with respect to myo-cardial function and heart injury and even attenuates kidney and liver dysfunction and tissue damage during well-resuscitated porcine septic shock.

Gordon et al.

.... Although these findings do not support the use of vasopressin to replace norepinephrine as initial treatment in this situation, the confidence interval included a potential clinically important benefit for vasopressin....
Barth et al. Effects of ventilation with 100% oxygen during early hyperdynamic porcine fecal peritonitis. *CCM* 2008;36:495


**Conclusions:** During early hyperdynamic porcine septic shock, 100% oxygen improved organ function and attenuated tissue apoptosis without affecting lung function and oxidative or nitrosative stress. Therefore, it might be considered as an additional measure in the first phase of early goal-directed therapy.

**Conclusions:** When compared with the previous report on hyperoxia initiated simultaneously with induction of sepsis, i.e., using a pretreatment approach, pure oxygen ventilation started when porcine fecal peritonitis-induced septic shock was fully developed proved to be equally safe with respect to lung function and oxidative stress, but exerted only moderate beneficial effects.


... total SOFA was significantly lower by day 7 (table 2) and the liver SOFA score was significantly lower from day 3 to day 7. ... In conclusion, in patients with septic shock, hyperoxia and hypertonic saline used as fluid resuscitation did not reduce the mortality at either 28 days or 90 days. ...
Osuchowski et al.
Minimum quality threshold in pre-clinical sepsis studies (MQTiPSS): an international expert consensus initiative for improvement of animal modeling in sepsis. *Intensive Care Med Exp* 2018;6:26

Zingarelli et al.

Libert et al.
Part II: Minimum Quality Threshold in Preclinical Sepsis Studies (MQTiPSS) for Types of Infections and Organ Dysfunction Endpoints. *Shock* 2019;51:23-32

Hellman et al.
Any questions?