Challenges for Identification of Pseudoanaphylactic Reactions in the Minipig – a Case Study

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Outline

• Background
• Study design
• Results
• Conclusion
Excipients - Background

• “excipere” to mount or to unhinge

• Everything in formulation except active drug; considered pharmacologically inert

• In pharmaceutical formulations to confer shape, volume, consistency of formulation to easily and properly deliver drug, modulate solubility and bioavailability, stabilize and preserve drug, improve drug taste etc.

• Surface active agents (surfactants): in biologics formulations to prevent physical damage during purification, filtration, transport, storage, delivery, to enhance stability, as wetting and emulsifying agents etc.

• Nonionic surfactants and other polymers typically heterogeneous; composed of a solution of many isomers and congeners
Selected nonionic surfactants

- **Poloxamer 188** (α-Hydro-ω-hydroxypoly(oxyethylene)poly(oxypropylene) poly(oxyethylene) block copolymer, Pluronic F68)
  
  ![Poloxamer 188 structure](image)

- **Polysorbate 20** (polyoxyethylene sorbitan monolaureate; E432)
  
  ![Polysorbate 20 structure](image)

- **Polysorbate 80** (polyoxyethylene sorbitan monooleate; E433)
  
  ![Polysorbate 80 structure](image)
## Maximum concentrations of surfactants used in clinical parenterals

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Recommended Range for Preclinical Use&lt;sup&gt;a&lt;/sup&gt; (oral and i.v.)</th>
<th>Frequency of Use in Commercial Formulations&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Concentrations Used in Some Commercial Preconcentrates&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Final Surfactant Concentration Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cremophor EL</td>
<td>5–10%</td>
<td>3</td>
<td>65% (Sandimmune)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>≤5% after a 20- to 100-fold dilution, i.v. infusion. Average volume per single dose = 3.5 ml&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>5–10%</td>
<td>1</td>
<td>51% (Taxol)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>≤10% after 5- to 20-fold dilution, i.v. infusion. Average volume per single dose = 25.3 ml&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cremophor RH 60</td>
<td>5–10%</td>
<td>22</td>
<td>50% (Vumon)</td>
<td>≤5% after a 10- to 100-fold dilution, IV infusion. 18% after a ~4-fold dilution, intravenous instillation</td>
</tr>
<tr>
<td>Tween 20</td>
<td>22</td>
<td></td>
<td>51% (Valstar)</td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>5–10%</td>
<td>72</td>
<td>100% (Taxotere)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>≤2% after a ~50- to 150-fold dilution, i.v. infusion</td>
</tr>
<tr>
<td>Soluplus HS-55</td>
<td>20–50%</td>
<td></td>
<td>50% (Pantol)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> % w/v on the basis of single 10 mg/kg excipient dose to a mouse or rat, from Li and Zhao (2007)

<sup>b</sup> From Nema and Brendel (2011).

<sup>c</sup> From Sturkley (2004).

<sup>d</sup> Significant evidence of local/systemic adverse reactions.

<sup>e</sup> From Gelderblom et al. (2004).

<sup>f</sup> It was not possible to identify the product.

<sup>g</sup> Licensed for use in Mexico.

Mainly Polysorbates 20 and 80 (up to 2%) used in clinical i.v. formulations

Williams et al., Strategies to address low drug solubility in discovery and development, Pharmacological Reviews, 2013
Non-IgE mediated hypersensitivity reactions of Poloxamer 188 and Polysorbates 20 and 80

**Poloxamer 188** (5 mg/mL) mediated complement activation (SC5b-9 levels) in serum from healthy humans (13 of 17 individuals)

Slow i.v. bolus of 50 mg/mL **Polysorbate 20** to dogs
- arterial blood pressure, heart rate, plasma fibrinogen ↓
- respiratory rate, lymph flow, and hematocrit ↑

Infusion of 5 mg/mL **Polysorbate 80** to dogs

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Moghimi SM et al. Causative factors behind Poloxamer 188 induced complement activation in human …. Biochim et Biophysica Acta, 2004
S Qiu et al, Complement activation associated with polysorbate 80 in beagle dogs; Int Immunopharmacol, 2013
Infusion-related complement activation by micellar drug carriers


- Maximum response in complement activation at ~30 min
- Surfactant concentration-dependent complement activation

Fig. 3. Time-dependence (A), and dose–response relationship (B) of complement activation induced by Cremophor-EL, Tween-80 and Tween-20 in normal human sera. In Panel A, sera were incubated in the presence of surfactants (1%, v/v) at 37 °C. In Panel B, sera were treated for 60 min at 37 °C. In both experiments, Zymosan A was used at 300 µg/ml as positive control. Bars represent mean ± SEM. (n = 3; *p < 0.05; **p < 0.01; ***p < 0.001).
Tolerability of Poloxamer 188 and Polysorbates 20 and 80

- FDA database: maximum potency (human) for P188 0.6% i.v.; PS 20: 2.4% i.v. and 0.2% s.c.; PS80: 50% i.v. and 0.3% s.c.

- Polysorbates regarded as nontoxic and nonirritant (moderately toxic by i.v.)
  LD50 i.v. mouse or rat >1g/kg

- Vitic excipient database (Lhasa Ltd.) – no information for i.v. or s.c. for PS 20 or PS 80

- Anecdotal reports
  - 30 min i.v. infusion 6.3 mL/kg of 19 mg/mL Tween 80 in 5% glucose with erythema, anxiety, itching on start of dosing, diminishing during infusion
  - Single 1.5 mL i.m. injection of Tween 20 (12 mg/mL) + other excipients well tolerated
  - 10 % DMSO 0.5 Tween 20 in saline with 0.2 % HCl to two minipigs with no adverse effects after a single i.v. dose at a pH >2.7
  - Limited experience, PK studies with Tween in formulations w/o abnormal clinical signs

- Minipigs are also refractory to anaphylactoid responses following intravenous injections of certain vehicles (e.g. Tween 80 and Cremophor EL).... [Link to blog post](http://www.criver.com/about-us/eureka/blog/august-2012/minipig-poised-to-replace-non-rodent-species-in-no) (Aug 2012)

http://www.accessdata.fda.gov/scripts/cder/iig/ (FDA database inactive ingredient search for approved drug products)
Handbook of Pharmaceutical Excipients; Rowe CR, Sheskey PJ, Cook WG and Fenton ME, 7th edition 2012
Strategies to address low drug solubility in discovery and development, Williams et al., Pharmacological Reviews, 2013
Complement activation-related pseudoallergy (CARPA)

- From simple discomfort to lethal reaction in man
- 4 characteristic symptoms in animal species: hemodynamic (blood pressure), hematologic (thrombocytopenia), biochemical (Ö CH50, Í TxA2) and skin (rash)

Study design – slow bolus intravenous study

0.1% Polysorbate 20
0.1% Polysorbate 80 - submicellar concentration
0.1% Poloxamer 188
- each at 1 mL/kg for 1 min on two consecutive days

non-naïve 3 + 3

Clinical Signs

ClinPath, Histamine, TBX2, SC5b-9
Histamine, TBX2, SC5b-9
ClinPath, Histamine, TBX2, SC5b-9
Histamine, TBX2, SC5b-9
Histamine, TBX2, SC5b-9
ClinPath, Histamine, TBX2, SC5b-9

BT, SpO2, heart rate
SpO2, heart rate
BT, SpO2, heart rate
BT
BT
Clinical Signs

predose
2 min
5 min
15 min
45 min
120 min
Complement-related side effects of drugs

Pre-clinical safety

- **Animal models for Complement activation-related responses:**
  - **Pigs / Dogs:** *(Szebeni, Am J Physiol Heart Circ Physiol 2006)*
    - Closely mimics CARPA symptoms (hemodynamic, cutaneous) and Cpt activation
    - Over-predictive in frequency
  - **Monkeys:** under-predictive *(Wassef Drug Delivery 1995)*
  - **Rats:** rather insensitive *(2-3 orders of magnitude less), never rash* *(Szebeni, J Lipos Res 2007)*

- **Investigative options:**
  - Interacting regions in C3 are evolutionary **highly conserved**, which allows for development of **C3-based pan-species** ELISA assay
  - Possibly more sensitive than CH50
Investigation of Complement activation

Selected approach

- 1 ml K₃ EDTA plasma, frozen on dry ice
- From dosed groups (no vehicle)
- Pan Specific C₃ Complement Reagent Kit (Quidel/TECO)

Designed and tested by Szebeni in vivo in pigs and rats IV injected with 0.1 mg/kg zymosan:
- Clinical symptoms induced in association with Complement consumption
- BUT high inter-animal and -species variability (e.g. x20 in pigs vs. x4 in minipigs)
Results

- Few drop outs (kinked or plugged catheter and catheter ripped out)
- Clinical signs (e.g. reddening of abdomen and/or head up to 15 min post-dose) in individual animals, likely due to struggling during dosing
- No effect on hematology, coagulation and clinical chemistry parameters
Complement activation SC5b-9 Levels
Thromboxane B2 concentrations
Histamine concentrations
Body temperature (inguinal)
Pulse rate (pulsoximeter)
Peripheral oxygen saturation (pulsoximeter)
Conclusion

• No obvious and clear signs of pseudoallergic reactions at submicellar concentration of 0.1% Polysorbate 20, Polysorbate 80 and Poloxamer 188 at 1 mL/kg for 1min given as slow i.v. bolus to minipigs

• Indications that tested excipients are well tolerated at submicellar concentrations of 0.1% (1 mg/mL)

• Improve quality of readouts for Complement assay:
  ᵃ CH50 remains reference tool
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