Reverse immunization with Doxebo turning tolerance to CARPA into a deadly reaction in pigs
Experience & Learning

1. CARPA project:
   a. Porcine and rat experiments
   b. ELISA testing for complement activation
   c. CLINAM -- European Summit for Clinical Nanomedicine

1. Cochleat project:
   a. Small Unilamellar Vesicle (SUV) Preparation
   b. Extrusion
   c. Dialysis
   d. AFM Imaging
   e. Phase Contrast Microscopy
   f. Fluorescent Microscopy
   g. Image Analysis
First.... What is CARPA?

Not a fish?
CARPA = complement activation-related pseudoallergy

- A type of allergic reaction hypersensitivity anaphylactoid reaction, infusion reaction, pseudo = non-IgE-mediated
- Main symptoms: heart and chest pain, rash, hypo/hypertension transient, reversible, but can be lethal in a small percentage
- A significant health care issue with hundreds of thousands reactions per year worldwide
- A major immune barrier of the intravenous application of nanomedicines, antibodies, micellar drugs, contrast media, proteins and enzymes
Current Prevention

Premedication
  steroids, antihistamines
Slow infusion
Exclusion of patients at risk
Desensitization of patients
  Example: Doxil reactions prevented by Doxebo
Porcine model of CARPA

- Bronchospasm
- Apnea
- Exhaled pCO₂ ↓
- Leukopenia
- Leukocytosis
- Thrombocytopenia
- Skin flushing & rash
- Left ventricular end-diastolic pressure ↓
- Cardiac output ↓
- Pulse pressure ↓
- Adenosine ↑
- Pulmonary hypertension with/without systemic hyper- or hypotension
- Tachycardia, bradycardia arrhythmias
- Thromboxane A₂ ↑
Pig laboratory in Herceghalom

Göttingen minipigs
Principle of desensitization: tachyphylaxis

- 0.01 mg/kg Doxil
- 0.5 mg/kg Zymosan

Blood pressure, % change

Minutes after start of injections
Prevention of Doxil reactions by desensitization with Doxebo in pigs

• Theoretical basis
  – tachyphylactic nature of liposome reactions
  – weak, subclinical reactions also can lead to tachyphylaxis

• Realization
  – slow infusion of low dose of Doxebo before treatment with Doxil (Caelyx)
Unclarified question regarding the use of Doxebo: window of time for tolerizing effect

Could it be applied once to remain effective over weeks?

Experimental test: Doxebo tretament 1 week before Caelyx treatment
Experiment

1. Slow infusion of Doxebo on day 0 in a dose that does not cause reaction

2. Repeating the same protocol 1, 2 and 3 weeks later. Doxebo infusion is followed bba treatment with Caelyx

1. Monitoring of
   1. hemodynamics,
   2. skin changes
   3. plasma IgM and IgG
   4. blood cell changes
   5. plasma markers of CARPA
Tests and blood samples

- Physiological endpoints
  - Weeks 0-3
    - Arterial BP from ear, ECG, exhaled CO2, respiration rate, body temp, skin
  - Week 4
    - PAP, SAP, ECG, exhaled CO2, respiration rate, body temp, skin
- Blood samples (at 0, 5, 10, 15 and 30 min post Doxebo or Caelyx
  - EDTA-containing plasma for
    - Anti-mPEG2000 IgG/IgM
    - blood cell counts
    - (+ indomethacin) TXB2
    - Other biomarkers (at least 4)
  - Hirudin-containing tubes for PAN assay
Reversal of the tachyphylactic effect of Doxebo 1 week after treatment

Reaction to Doxebo and huge reaction to Caelyx instead of lack of reaction (tachyphylaxis)
Amplification of CARPA caused by Caelyx by Doxebo pre-treatment 1 week before

Huge reaction instead of lack of reaction
Symptoms

- Major flushing through all 3 injections
- Massive rise in HR, followed by prolonged period of heightened levels.
- Massive rise and then drop in SAP, followed by leveling off.
Explanation of tachyphylaxis reversal: immunization

- Massive formation of anti-liposome IgM and IgG provide evidence for immunization
- Finding proves the Doxebo is immunogenic
- This effect is luckily avoided in the clinics without foreknowledge due to the standard Doxil injection schedule of 3 weeks, as well as the immunosuppressive nature of Doxilrubicin.

Blue, red and green: different animals

ELISA determination of anti-liposome immunoglobulins

Weeks after doxebo treatment

Measurements of Gergely Kozma
Historical Analogy: 1941 re-written...

• Japan bombs Pearl Harbor...
• Leads to initial US military vulnerability.
• US responds by stockpiling thousands of nuclear weapons.
• US and Japan sign a one week ceasefire.
• The wind deviates a small Japanese weather balloon a little bit in what seems like the direction of Pearl Harbor and...
• US releases so many atom bombs on Japan that within minutes the atmosphere of Earth becomes uninhabitable to all life.
Conclusions

- Doxebo is immunogenic
- Infusion schedule is critical: Doxebo needs to be applied soon before treatment with Doxil/Caelyx
- If applied 1 week before treatment, it can amplify the liposome reaction, instead of preventing it. Hence, individuals receiving Caelyx are particularly vulnerable to CARPA reactions within a week after initial infusion of Doxebo, (even if initial infusion caused no reaction).
- Blood tests for immunization (particularly buildup of IgM) will be recommended for patients receiving Doxebo
- The model represents „immunization to anaphylaxis”
- The model may be developed to immunization to anaphylactic shock & death
- The model can be developed to be a sensitive quantitative model of immunogenicity to nanomedicines, which is an unmet medical need
Questions for further research...

• Does it exist and what are the factors determining immunogenicity of Doxebo in humans?
• How does immunization to one nanomedication effect the body’s reactogenicity to other nanomedications?
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