## CARPA Diem!

# Reverse immunization with Doxebo turning tolerance to CARPA into a deadly reaction in pigs

Semmelweis - Princeton 2015 Internship Program

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### **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 activationrelated pseudoallergy

- A type of allergic reaction hypersensitivity anaphylactoid reaction, infusion reaction,
  - pseudo = non-lgE-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**



### **Pig laboratory in Herceghalom**





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#### **Principle of desensitization:**

tachyphylaxis 0.01 mg/kg Doxil 0.5 mg/kg Zymosan 200 -PAP 180 -SAP Blood pressure, % change 160 0.01 mg/kg Doxil 140 120 100 80 60 40 20 0

**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 lgG/lgM
    - 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 hightened levels.
- Massive rise and then drop in SAP, followed by leveling off.



# Explanation of tachyphylaxis reversal: immunization

![](_page_15_Figure_1.jpeg)

Weeks after doxebo treatment

- 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 immunosupressive nature of Doxilrubicin.

### 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.

![](_page_16_Picture_7.jpeg)

#### **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 imunization (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?

#### Special thanks to...

Dr. Rudolf Urbanics Dr. Domokos Csukas Dr. Réka Nemes Prof. Miklós Kellemayer Dr. Tamás Bozo Ms. Luisa Duarte-Silva

#### And...

#### Prof. János Szebeni