Venoms and antivenoms
DEFINITIONS

- Purposes of the venom
  - Immobilization of the prey (replaces the limbs)
  - Digestion of the prey
  - Defense?

- Chemical composition according to the purpose

- Toxicity according to the purpose
Causes of venoms variations

• Animal
  – Species
  – Venom variation

• Experimental conditions
  – Technical procedures

• Individual (or patient)
Echis ocellatus

12.5% reducing SDS-PAGE, 29 µg/lane
Bitis arietans

12.5% reducing SDS-PAGE, 15 µg/lane
Synthesis of the venom (after Bdolah, 1979)

Mg or mg/ml

Days after milking

R² = 0.9262

R² = 0.9175

- Protein concentration (mg·ml⁻¹)
- Venom yield (mg)
Equivalence triangle of toxicity

**VENOM**

**ANIMAL MODEL**

**PHYSIOLOGICAL EFFECTS:**
The venom components should have toxic effects disclosed in the animal model

**PHARMACOLOGICAL EQUIVALENCE**

**TOXICOLOGICAL EQUIVALENCE:**
The animal model should be sensitive to the same toxic components that the victims

**PATIENT**

**PHYSIOLOGICAL EFFECTS:**
The venom components should induce identical symptoms in envenomed patients
Mode of action of the venoms

1. Toxins  \(\rightarrow\)  Dose-dependent effects
   - Cells
   - Nervous system

2. Enzymes  \(\rightarrow\)  Time-dependent effects
   - Blood coagulation
   - Inflammation
   - Cellular digestion $\Rightarrow$ necrosis
Neuromuscular junction

1. Presynaptic terminal
2. Sarcolemma
3. Synaptic vesicle
4. Nicotinic acetylcholine receptor
5. Mitochondrion
1. Ion channel linked receptor
2. Ions
3. Acetylcholine (or other ligand)
Mode of action of neurotoxins

- **β-neurotoxin**: Calciseptin blocks A-Ch liberation
- **Dendrotoxin**: Facilitate A-Ch liberation
- **Fasciculin**: Inhibits acetylcholinesterase
- **α-neurotoxin and κ-neurotoxin**: Muscarinic neurotoxins

Diagram:
- Nerve Fiber
- Muscle Fiber
- Calcium ions (Ca++)
- Potassium ions (K+)
- Sodium ions (Na+)
- A-Cholinesterase
Mode of action of snake venoms on blood coagulation

- **Prothrombinase**
  - $\text{X + V + Ca}^{++} + \text{Phospholipides}$

- **Platelets**
  - Thromboplastin (X)
    - $\text{+ VII + Ca}^{++}$

- **Prothrombin (II)**
  - $\text{XII + XI + IX + Ca}^{++}$
  - PROTHROMBINASE
    - $\text{X + V + Ca}^{++} + \text{Phospholipides}$

- **Thrombin (IIa)**
  - **Fibrinogen (I)**
    - Soluble FIBRINE
      - XII (= FSF)
      - Stable FIBRINE (= clot)

- **Plasminogen**
  - PLASMIN
    - CLOT DISOLUTION

**Disintegrins**

**Phospholipases A$_2$**

**Prothrombin activators**

**Thrombin-like enzymes**

**Proteases**

**Plasminogen activators**
Bleedings and hemorrhages

1. Local bleedings (hemorrhagins)

2. Systemic bleedings (hemorrhagins)

3. Systemic bleedings (afibrinogenemia)
Basic approaches of snakebite treatment

1. Symptomatic treatments

2. Venom neutralisation:

   A. Antidote
      - inhibition/destruction of the venom
      - competition
      - antagonism

   B. Immunotherapy
      - stimulation of the defenses
      - etiologic (specific) = antivenom
Mechanic extraction of the venom
Mechanic extraction of the venom

Alberts et al., 2004

Radioactive mock venom

<table>
<thead>
<tr>
<th>Injected venom</th>
<th>Remaining venom</th>
<th>Unexplicated losses</th>
<th>Extracted venom</th>
<th>Losses from the wound</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Increased of the edema after inoculation of Crotalus atrox venom in a porcine model

Bush et al., 2000
In this study, healing around the puncture site was similarly delayed in pigs with extractor lesions, which took at least 3 weeks to heal.
Limitations of the extraction or destruction of the venom

• Quick diffusion of the venom

• Competition between the venom and other body fluids

• Iatrogenic or unexpected effect risks
ANTIDOTES

• Systemic action (against venom activities)
  • atropine (antagonist of Dendrotoxins and Fasciculins)
  • Competition on action site (Securidaca longepedunculata)
  • immunostimulation (Rauvolfia, Valeriana, sarsaparilla)

• Specific action (inhibition of venom components)
  • anti ATPase (Gymnema sylvestre)
  • anti Plase A₂ (Aristolochia sp.)
  • Hemorrhagins
## ANTAGONISM - POTENTIATION

<table>
<thead>
<tr>
<th>Extracts administered</th>
<th>LD$_{50}$</th>
<th>ED$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Schummaniophyton magnificum</em></td>
<td>2</td>
<td>1,5</td>
</tr>
<tr>
<td><em>Bidens pilosa</em></td>
<td>2</td>
<td>1,2</td>
</tr>
<tr>
<td><em>Garcinia lucida</em></td>
<td>1,5</td>
<td>2</td>
</tr>
<tr>
<td><em>Securidaca longepedunculata</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Hemidesmus indicus</em></td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Mode of action of *Securidaca*

- **Acetylcholine Receptor**
  - $\text{Na}^+$
  - $\text{K}^+$

- **Securidaca**

- **Acetylcholine**
  - $\text{Na} \cdot \text{K}^+$

- **Neurotoxin**
  - $\text{K}^+$

- **Acetylcholine Neurotoxin**
  - $\text{Na} \cdot \text{K}^+$
Mode of action of *Securidaca*

**Securidaca**

**Acetylcholine**

**Neurotoxin**

*Na* • •

*Na* • •

*K* • •

*K* • •
Antivenom manufacture

• Passive immunotherapy: fabrication of antibodies by an animal
• Horse (Equidae)
• Goat – Rabbit – Camel – Bird
• Immunisation protocol
• Plasma and IgG purifications
• In vitro / in vivo control tests
Immunotherapy = Antivenom

1. The only etiological antivenomous treatment

2. Unjustified poor reputation

3. Undeniable efficacy if appropriate antivenom

4. Confirmed safety with current products
Structure of immunoglobulins G (IgG)
Enzymatic digestion

pepsin

papain

papsaïne

F(ab')

Fc'

pFc'
Antivenoms mechanism of action

1. Antibodies $\rightarrow$ specifically recognizes antigen

2. Recognition $\rightarrow$ precipitation

3. Precipitation $\rightarrow$ complete elimination from the body

Precipitation $\neq$ neutralization
Expected benefits of the antivenoms

Efficacy
  Systemic and local

Tolerance - safety
  Immediate adverse effects
  Delayed adverse effects (serum sickness)

Stability
  Duration and climatic conditions

Accessibility and affordability
  Cost
  Available in isolated centers

Theakston RDG *et al.* *Toxicon*, 2003, 41: 541-557
Passive immunotherapy

I - Efficacy

1. Animal immunization:
   - Immunization strategy
   - Choice of venoms

2. Choice of IgG Fragments

3. Administration by intravenous route

4. Dosage:
   - Loading dose or effective lowest dose level
   - According to antivenom titer

5. Influence of delaying of treatment
Distribtion of the venom and antivenom in the organism compartments (from Ismail et al., 1996)

Compartments

**Shallow tissues**
- Toxins = 1
- Fab
- IgG
- F(ab’)2
- Venoms:
  - N. melanoleuca = 0,5
  - N. haje = 1,7

**Blood**
- Toxins = 1
- IgG
- F(ab’)2
- Fab
- Venoms = 1

**Deep organs**
- Toxins = 1,7 à 3,8
- Fab
- IgG
- F(ab’)2
- Venoms:
  - N. melanoleuca = 3,7
  - N. haje = 1,4
Pharmacokinetics of venoms and antivenoms (from Rivière et Audebert, 1997; 1998)

Venom (mg/l)

- Antivenom administration
- Control
- F(ab’)2
- Treated rabbits
Antivenom dose effects
(from Rivière et al., 1997)
Antivenom delay effects
(from Rivière et al., 1997)

Venom (ng·ml⁻¹)

Time (hours)

AV administration

Control (1 LD₅₀)
3 hours (1 ED₅₀)
7 hours (1 ED₅₀)
24 hours (1 ED₅₀)
Cross reaction inside the same genus

Viperidae

Echis
Paraspecificity of African Echis species

- E. leucogaster
- E. ocellatus
- E. pyramidum

% of homologous proteins against:

- E. leucogaster
- E. ocellatus
- E. pyramidum

Antiserum against
Cross reaction inside the same genus

Elapidae

Dendroaspis (Mambas)
Paraspecificity of Mamba venoms

- D. angusticeps
- D. jamesoni jamesoni
- D. jamesoni kaimose
- D. polylepis 218.020
- D. polylepis 908.020
- D. polylepis KZoo
- D. viridis LTXN

% of homologous

189 D. ang LTXN
190 D.j.j. LTXN
191 D. pol. KZoo
193 D. vir LTXN

Antiserum against
Expression of the neutralizing potency

1. $\text{ED}_{50}$ ($\mu l$ of antivenom neutralizing 3 $\text{LD}_{50}$)
   
   Mix of variable quantity of antivenom and fixed quantity of venom killing 50% of mice

2. Weight of dry venom (mg of venom/ml of antivenom)
   
   Ratio of the amount of venom mixed with 1 ml of antivenom able to kill 50% of mice

3. Number of $\text{LD}_{50}$ neutralized by 1 ml of antivenom
Comparison between $ED_{50}$ et Weight of dry venom

$DE_{50}$ ($\mu l/5DL_{50}$) vs mg/ml

Species: Va ammodytes, Va aspis, Vb berus, Mo xanthina, D deserti, Ma schweitzeri
Thank you

It doesn't matter that we're not venomous:
Most people stay well clear of us anyway...