Therapy of Nerve Agent Poisoning – Up-date and New Approaches

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Toxicology Symposium of the Low Lands

9th June 2017
Ghent, Belgium
Nerve Agents and other Organophosphorus Compounds

**G - Agents**

Tabun (GA), Sarin (GB), Cyclosarin (GF), Soman (GD) (and Analogs)

**V - Agents**

VX, Russian VX (VR), Chinese VX (CVX) (and Analogs)

**OP - Pesticides**

Diethyl-type, Dimethyl-type, hundreds of other compounds!
Characteristics of Poisoning by Nerve Agents

Generally assumed: patients exposed up to $5 \times \text{LD}_{50}$

Inhalative G-Type Nerve Agent Poisoning
- Immediate onset of cholinergic crisis
- Short persistence of nerve agent in the body

Percutaneous V-type Nerve Agent Poisoning
- Prolonged onset of cholinergic crisis
- Persistence of nerve agent over several days in the body
Effects of Organophosphorus Compounds

Mechanism:
- inhibition of AChE
- accumulation of ACh
- disturbance of cholinergic functions
# Clinical Challenge of Nerve Agent Poisoning

## Signs and symptoms

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Nerve agent poisoning</th>
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<tbody>
<tr>
<td></td>
<td>Slight</td>
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<td>Miosis / lacrimation</td>
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<td>Local fasciculations</td>
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<td>Hypersalivation / sweating</td>
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<td>Nausea</td>
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<td>Vomiting / defacation / emicition</td>
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<td>Bronchoconstriction / bronchorrhoe</td>
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<td>Bradycardia / circulatory depression</td>
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<td>Respiratory depression</td>
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<td>Convulsions</td>
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<td>Coma</td>
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Worek et al. Arch Toxicol 2016
Effects of Organophosphorus Compounds

Mechanism:
- inhibition of AChE
- accumulation of ACh
- disturbance of cholinergic functions

Life threatening effects:
- bronchoconstriction/
  bronchorrhoea (muscarine receptors)
- central respiratory arrest (muscarine and nicotine receptors)
- peripheral respiratory arrest (nicotine receptors)
ChE Check Mobile for on-site Confirmation of the Clinical Diagnosis

Acetylthiocholine → AChE → BChE

Acetate → Thiocholine + DTNB → 5-Mercapto-2-nitrobenzoate + TNB⁻

Ellman et al. Biochem Pharmacol 1961
Biomedical verification of exposure to chemical agents

- chemical warfare agents
- degradation products
- metabolites and adducts
Peculiarities in Treatment of Chemical Agent Poisoning

Self – Protection; Working under aggravated conditions;
Protection of medical units; Latency period.
Decontamination of victims with signs and symptoms

Atropine for Nerve Agent Poisoning

Military doctrine: autoinjectors for self and buddy aid

Enhanced first aid and clinical care by medical personnel

Aggressive atropine dosing

Recommended German regimen
2 mg, i.m. followed by consecutive doubling the dose (4 – 8 – 16 - 32 mg) and finally infusion at field hospital

Criteria
Clear chest on auscultation; heart rate >80 beats/min; pupils no longer pinpoint; dry skin (axilla); systolic blood pressure >80 mmHg

Development of Oximes

Synthesis of thousands of oximes since the early 1950ies
- Monopyridinium oximes
- Bispyridinium oximes
- Asymmetric oximes
- Substituted pyridinium oximes
- Uncharged oximes

2017
Oximes in use or in advanced development

- 2-PAM 1955
- TMB-4 1958
- MMB-4 1959
- Obidoxime 1959
- HI-6 1968
Effects of Obidoxime in a Patient with Parathion Poisoning

**Patient:** 56-year old, female

**Emergency situation:** Unconscious, cardiovascular resuscitation

**Clinical course:** As the clinical course situation remained critical for about 4 days without obidoxime, the regimen was started

**AChE activity of red blood cells**

**Neuromuscular transmission**

prior to Obidoxime

after Obidoxime
Reactions Occurring at AChE with an OP and an Oxime

\[
\begin{align*}
\text{OP} & \quad \text{enzyme} \quad \text{leaving group} \quad \text{inhibited enzyme} \\
\begin{array}{c}
\text{X} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{P} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \end{array} & + \ A\text{ChE-OH} & \xleftrightarrow{} & \ X & + \ A\text{ChE-O-PO-} \\
\begin{array}{c}
\text{H}_{2}\text{O} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{R} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{N} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \end{array} & \quad \text{R} \ = \ \text{NOH} & \quad \text{H}_{2}\text{O} & \quad \text{AChE-OH} \\
\begin{array}{c}
\text{CH}_{3}-\text{CH}_{2}\text{OH} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \end{array} & + \ A\text{ChE-OP-OH} & + \ A\text{ChE-O-PO-} \\
\begin{array}{c}
\text{H} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{P} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{O} \end{array} & \quad \text{H} \ = \ \text{O-PO-} \\
\begin{array}{c}
\text{AChE-OH} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \\
\text{R} \ = \ \text{NOH} & \quad \text{H}_{2}\text{O} & \quad \text{AChE-OH} \\
\text{CH}_{3}-\text{CH}_{2}\text{OH} & + \ A\text{ChE-OP-OH} & + \ A\text{ChE-O-PO-} \\
\text{H} \ = \ \text{O-PO-} & \quad \text{R} \ = \ \text{NOH} & \quad \text{H}_{2}\text{O} \\
\text{AChE-OH} \quad \text{AChE-OH} & \quad \text{oxime induced} & \quad \text{aging} & \quad \text{spontaneous reactivation}
\end{align*}
\]
Kinetic-Based Computer Model

\( k_i \)  
\( [E] + [OP] \rightarrow [EP] \)  
(1)

\( k_a \)  
\( [EP] + H_2O \rightarrow [EP'] \)  
(2)

\( k_s \)  
\( [EP] + H_2O \rightarrow [E] + [P] \)  
(3)

\( k_r \)  
\( K_D \)  
\( [EPOX] \rightarrow [E] + [POX] \)  
(4)

\[ k_{obs} = \frac{k_r \cdot [OX]}{K_D + [OX]} \]

\[ \frac{d[E]}{dt} = -k_i \cdot [OP] \cdot [E] + k_s \cdot [EP] + k_{obs} \cdot [EP + EPOX] \]

\[ \frac{d[EP]}{dt} = k_i \cdot [OP] \cdot [E] - k_s \cdot [EP] - k_{obs} \cdot [EP + EPOX] - k_a \cdot [EP] \]

Pharmacokinetics

Toxicokinetics

Worek et al. TAP 2005
The Use of Kinetic Constants, Pharmacokinetic and Toxicokinetic Data for Prediction of AChE-Activity in Human Poisoning

Patient:
A 45-year old, male

Emergency situation:
Unconscious, severe signs and symptoms of cholinergic crisis. 1.5 mg of atropine, intubation and initiation of artificial ventilation.

Clinical course:
2 bolus doses of obidoxime together with an atropine infusion at the local hospital. Transfer to the ICU of Technical University, Munich. The patient recovered uneventfully.

Worek et al. TAP 2005
Reactivation of OP Inhibited AChE with Oximes

Calculated Plasma Concentration of Oximes after i.m. Injection

Obidoxime

Pralidoxime

Translation of Clinical Findings from Human Poisoning to an Animal Model

Poisoning by dimethoate (oral) and treatment with 2-PAM

Worek et al. Chem Biol Interact. 2010
Is there a Correlation between RBC-AChE-Activity and Clinical Status?

A parathion poisoned patient was treated with obidoxime. The cholinesterase status and NMT were monitored during treatment at the ICU.

Eyer et al. Clinical Toxicology 2009
Patient-Oriented Therapy by Using the Cholinesterase Status

Cholinesterase Status:
- AChE and BChE activity
- Reactivatability of AChE
  - Reactivatability at all
  - Aging
- Persistence of poison load

Treatment:
- Appropriate oxime
- Oxime as long as substantial reactivation may be expected
  - Given reactivatability
  - Persistence of active poison
- Oxime stop:
  - Reactivation achieved and no poison load
  - Complete aging
  - Increase of BChE activity
Translation of Findings from OP-Pesticide-Poisoning to Nerve Agent-Poisoning

Male York-Landrace cross swine (about 20 kg)

**Poisoning:**

3 x LD$_{50}$ VX, p.c.

**Treatment:**

HI-6 (12.7 mg/kg) / Atropine sulfate (0.05 mg/kg) i.m., according to signs and symptoms

**Field laboratory diagnosis:**

AChE activity on-site

**Plasma sampling for laboratory analysis:**

HI-6, VX
Treatment of a Percutaneously VX Poisoned Pig
Alternative Approaches for Therapy of Nerve Agent Poisoning

Enhanced elimination by scavengers

Cyclodextrins
Cyclodextrines as Small Molecular Scavengers in Nerve Agent-Poisoning

2-O-(3Carboxyl-4-iodosobenzyl)-β-cyclodextrin

Anesthetized (Medetomidine – Fentanyl – Midazolam)
Cannulated A. carotis and V. jugularis
ß-CD (6-OxP-CD; 100 mg/kg i.v. at -5 min)
Cyclosarin (100 µg/kg s.c.; ~2LD50 at 0 min)
No post-exposure treatment

Treatment of a GF Poisoned Guinea Pig with 6-OxP-CD

Worek et al. Toxicol Lett 2014
Cyclodextrines as Small Molecular Scavengers in Nerve Agent-Poisoning

2-O-(3Carboxyl-4-iodosobenzyl)-β-cyclodextrin

VX
Tabun
Sarin
Cyclosarin

Sulfonatocalix[4]arenes as Small Molecular Scavengers in VX-Poisoning

Calixarene-derivates were decorated with substituents in order to achieve fast detoxification in water.

Detoxification of VX with Calixarenes containing hydroxamic acid was about 3500 times faster when compared with spontaneous hydrolysis.

Schneider et al. Angew Chem Int Ed Engl 2016
Treatment of VX Poisoned Guinea Pigs with PTE C23

Anesthetized (Medetomidine – Fentanyl – Midazolam)
Cannulated A. carotis and V. jugularis
VX (18 µg/kg s.c.; ~2LD50 at t = 0 min)
PTE C23 (5 mg/kg at t = 5 min)
No post-exposure treatment

18 µg/kg VX s.c. (~2LD50)

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<th>0 - 20</th>
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<th>41 - 60</th>
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18 µg/kg VX s.c. (~2LD50) followed by 5 mg/kg PTE i.v. after 5 min

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Worek et al. Toxicol Lett 2014
Oximes in OP-poisoning – Hope and Despair

Alternative Approaches for Therapy of Nerve Agent Poisoning

- Enhanced elimination by scavengers
  - Cyclodextrins
- Modulation of nicotinic ACh-receptors
  - Enzymes, e.g. PON1, PTE

Wille et al. Tox Lett, 2016
Restoration of Nerve Agent Blocked Muscle Force

Human intercostal muscle

Horizontal 4-chamber-organ bath with stimulation-electrodes

Muscle-force after electrical field stimulation (25 Hz)

Seeger et al. Toxicology 2012
Interaction of MB 327 with Human Nicotinic Acetylcholine Receptors (α7)

Functionality: Whole cell recording by automated patch clamp platform

![Graph showing current-voltage relationship with different concentrations of agonists and modulators.]

Agonist

- Nicotine

Modulators

- PNU 120596
- MB327

Scheffel et al. 2017 to be published
Interaction of MB 327 with Nicotinic Acetylcholine Receptors (Muscle-type)

Membrane preparation of *Torpedo californica* electric organ (muscle-type nAChR)

- Affinity on \([^3]H\)epitatidine binding sites
- Functionality based on solid supported membranes (SSM)

Therapeutic Approach in Nerve Agent-Poisoning

Self protection

Prompt reactivation of inhibited AChE
  - even in the absence of severe signs and symptoms
  - prolonged oxime treatment

Atropine for muscarinic signs and symptoms

Benzodiazipines for treatment and/or prevention of seizures

Supportive therapy
  artificial ventilation, sedation, cardiovascular stabilisation
Outlook: Alternative Therapeutic Approach in Nerve-Agent Poisoning

Binding and enhanced elimination by scavengers:

- small molecular scavengers
- human BChE / & oxime
- human AChE / & oxime
- PON1; PTE

Receptor active compounds
Summary and Recommendations

Don’t wait for symptoms to develop!

Determine AChE activity in case of possible exposure as soon as possible!

Administer atropine according to signs and symptoms!

Administer an effective reactivating oxime when AChE activity has dropped even in the absence of clinical signs and symptoms!

Maintain oxime therapy as long as the organophosphorus compound is persisting in the body!
Acknowledgement

R. A. Hatz  M. Eichhorn  M. Lindner
J. E.H. Tattersall  C.M. Timperly  A.C. Green  M. Bird
D. Kiderlen  K. Wanner  B. Fichtl  T. Wein  P. Eyer  S. Rappengluck  M. Radtke

J. Mikler  I. Hill  K. Weatherby
D. Tawfik  M. Goldsmith  Y. Ashani  J.L. Sussman
K. Niessen  G. Reiter  T. Wille  G. Heyes  T. Hannig  S. Eckert  N. Herkert  S. Muschik  C. Scheffel
T. Seeger  H. John  M. Koller  G. Engl  S. Kirchner  S. Kämpfer  L. Schnitzler  N. Boos  C. Wübbeke

S. Kubik  M. Zengerle  A. Bierwisch  C. Schneider
M. Eddleston

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Thank you for your attention