Acetylcholinesterase Inactivation and Reactivation: A Tale of Two Consequences

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Aging → Alzheimer's disease → Molecular and Cellular changes → Cognitive Decline
Catalysis, Inhibition and Reactivation of Acetylcholinesterase

A. Association of ACh

B. Edrophonium complex

C. Reversible neostigmine binding

D. Reversible DFP binding

E. Reactivation of DFP-AChE by 2-PAM
Acetylcholine at the Base of the Active Center Gorge
Cholinesterase Inhibitors

• Produce only a short lived plateau (6-12 months)
• Increasing the dose enhances side effects
• Little enhancement with paired agents (donepezil and memantine)
• Balance of benefit and risk
  a. Quality of life
  b. Caregiver relief
  c. Pharmacoeconomics—Cappell et al., CNS Drugs 24: 909-927 (2010)
Structures of Cholinesterase Inhibitors

Donepezil and Galantamine are reversible inhibitors, while Rivastigmine is a carbamylating agent (slowly reversible)
Properties of an Ideal Cholinesterase Inhibitor

- CNS activity exceeds peripheral autonomic and motor activities. *(partial success)*
- No liver toxicity as seen with Tacrine (tetrahydroacridine) *(Success)*
- Devoid of peripheral side effects *(Lack of Success)*
Side Effects

• Gastrointestinal Disturbances: nausea, vomiting, hyper-motility
• Visual field limitations; meiosis
• Insomnia-long acting
• Muscle tremors and fasciculations
• “Cholinergic Crisis”
“Cholinergic Crisis” Results in

• Meiosis (intense pupil constriction) in the absence of a sympathetic reflex response.
• Brow pain
• Excessive gastrointestinal activity
• Muscle tremors and fasciculations
• In the extreme--convulsions
Why do we see greater efficacy in Parkinson’s Disease (PD) than Alzheimer’s Disease (AD)?

• Balance of dopaminergic and cholinergic stimulation in PD allows for a greater window for therapeutic endpoints.

• Multiple transmitters responsible for Alzheimer’s dementias --cholinergic pathways are primarily presynaptic, releasing other transmitters

• Nigrostriatal pathway is more discrete in PD etiology.

Reactivation Mechanisms and Tissue Disposition Affecting Efficacy of Oximes in Averting Toxicity from Organophosphate Exposure

Sarin Experiences Japan: 1994-1995; Arum Cult
Matsumoto -Outdoor Exposure
Tokyo Subway- Controlled Ventilation System
Sarin Experience Syria: Despotic and Inhumane Leader
Up to 1,000 deaths and 3,000 toxic events
Concepts that Require Continuing Re-evaluation

• Sarin Use (Vapor pressure and dispersion-Partial pressure latency of action distal to ground zero.

• Distinctions of Pre- and Post-exposure Antidotes versus Scavenging Agents (Applicability in in vivo, field situations?)

• Importance of Studies in Multiple Animal Species (double jeopardy for toxicant-antidote combinations in FDA Animal Rule)

• Value and Limitations of Molecular-based Techniques: Capture the transition state)

• Requirements for Parallel Pharmacokinetic, Toxicity and Efficacy Studies for Acute Exposures.
Catalysis, Inhibition and Reactivation of Acetylcholinesterase

A. Association of ACh

B. Edrophonium complex

C. Reversible neostigmine binding

D. Reversible DFP binding

E. Reactivation of DFP-AChE by 2-PAM

- **carbon**
- **oxygen**
- **nitrogen**
- **hydrogen**
- **phosphorus**
- **fluorine**
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\[ V \approx 300 \text{ Å}^3 \]
RS194B + VX inhibited AChE
In vitro Reactivation Constants for Recovery of AChE Activity

**Hydroxyimino acetamide Alkyl amines**

<table>
<thead>
<tr>
<th>oxime</th>
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<th>$K_{ox}$</th>
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**Reference Compounds**

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$$k_r = k_2 / (1 + K_{ox} / [oxime])$$

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![AChe reaction diagram](https://via.placeholder.com/150)
pH Dependences of Oxymolysis of VX in Buffer (Top) versus Oxime Catalyzed VX Conjugates with AChE Serine (Bottom)
Reaction steps in nucleophilic reactivation of OP-AChE with an oxime (RCH=NOH). $K_{ox}$ is a Michaelis type constant for formation of sarin-AChE*oxime reversible complex that is practically identical to the equilibrium dissociation constant, since the maximal phosphorylation rate constant ($k_2$) appears much slower in vast majority of cases than the dissociation rate constant of the complex.
Oxime-Amine Zwitterion with Ionizing Species

\[
\begin{align*}
\text{RS194B} & \quad \text{pK}_{a(NH^+)} = 8.8 \\
pK_{a(NO)} & \quad = 8.7
\end{align*}
\]
Reactivation of native peripheral AChE
Oxime-assisted Catalytic OP hydrolysis in Plasma
Oxime Reactivation of native AChE in CNS

Blood-Brain Barrier

Reactivation of native peripheral AChE
Oxime-assisted Catalytic OP hydrolysis in Plasma

Alveolar Membrane

Site of Exposure
Superiority of RS 194B-Plasma and Tissue Kinetics
Plasma in Mice: dashed line Brain: solid lines

[Graph showing the concentration of RS194B and RS41A over time in plasma and brain samples.]

RS194B
RS41A
Plasma Levels after Intravenous, Intramuscular and Oral Dosing in Mice

PK profiles of RS194B in mouse plasma following various routes of administration to the mouse: i.v.-20 mg/kg; p.o., 50 mg/kg; p.o. 200 mg/kg, i.m. 80 mg/kg; The data show rapid oral absorption within 20 min and bioavailability >50%.
Plasma and Brain Concentrations After an i.m. Loading Dose Followed by Four Oral Maintenance Doses in Mice
RS194B and 2PAM Protective Indices upon i.m. and p.o. (gastric lavage) Administration in Mice

<table>
<thead>
<tr>
<th>Protective Index</th>
<th>1 min after VX i.m.</th>
<th>15 min before VX p.o.</th>
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<td>⇒ ( \frac{(OP , LD_{50} , with , oxime)}{(OP , LD_{50} , without , oxime)} ) _ oxime _</td>
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<td>42 mg/kg p.o.</td>
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Oxime doses set as 25% of individual oxime LD\(_{50}\) for i.m. administration.
FDA Animal Rule for Toxicants and Antidotes

- Mode of Administration
- Pulmonary design and animal size
- Pulmonary physiology in non-human primates and rodents.
- Olfactory system dependence-turbenate structures
- Fraction of cardiac output from absorption point
- Sequelae of cholinergic symptoms
Disposition of Antidote from Absorption to the Lung
Recovery of Macaque Blood Cholinesterase Activities from Sarin
Yvonne Rosenberg, PlantVax; Tom Snider, Battelle Institute

- **AChE**
  - 0.5 LD50
  - 1.0 LD50
  - 1.0 LD50 + Oxime

- **BChE**
  - 0.5 LD50
  - 1.0 LD50
  - 1.0 LD50 + Oxime

*Hours Post-GB*
Recovery of Macaque Blood Cholinesterase Activities after Sarin Exposure (Top) AChE; (Bottom) BChE (Tom Snider, Battelle; Yvonne Rosenberg, PlantVax)

Oxime (RS194B) administered at 62.5 mg/kg and Atropine 0.28 mg/kg 2.75 min after a 31 min sarin exposure. BChE pre-administered by inhalation to obtain 6.0 mg/kg deposited. Green monkeys survived, red and orange monkeys died.
Pharmacokinetics, Toxicity & Disposition

Jeremiah Momper (UCSD)
Danielle Hagstrom (UCSD)
Eva-Maria Collins (UCSD)
Yvonne Rosenberg (PlantVax)
Don Blumenthal (Utah)
Ben Capacio (ICD)
Al Ruff (ICD)
Erica Fradinger (Whittier College)
Hayden Schmidt (Whittier College)
Zrinka Kovarik (IMROH, Zagreb)
Suzana Berend (IMROH, Zagreb)

Chemical Design & Synthesis

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Rakesh Sit (TSRI)
Zoran Radic’ (UCSD)

AChE Structure-Function

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William Hou (UCSD)
Gabi Amitai (IIBR-Weizmann, Israel)
Michal Harel (Weizmann, Israel)

AChE Crystal Structure

Zoran Radic (UCSD)
Pascale Marchot (Marseille)
Yves Bourne (Marseille)
Andrii Kovalevsky (ORNL)
Xiaolin Cheng (ORNL)
NASA Space Station
Gratitude and Recognition of the Many Students and Fellows from Yokohama City University Who Trained at UC San Diego

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