Eminent Toxicologist Lecture Series
Pesticide Neurotoxicity – More or Less

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Scope of Toxicology

• Toxicology studies the effects of chemicals on biologic organisms.

• Many disciplines contribute to toxicology. There are many types of toxicologists and they have many different backgrounds.

• Toxicology can contribute to other biomedical disciplines.

Frumkin, Environmental Health, 2010
Examples of Classifications of Toxic Substances

• By chemical class and/or use
  • Solvents, pesticides, heavy metals, etc.

• By target organ or physiological system affected
  • Nervous system, immune system, liver, etc.

• By type of adverse effect
  • Neurotoxicity, developmental toxicity, etc.

• This presentation will deal with pesticides and the nervous system
Defining “Pesticides”

• Agents used against any unwanted living organism
  • Insecticides
  • Rodenticides
  • Herbicides
  • Fungicides

• Beneficial: economic; nutrition and health

• Concerns: toxicities, residues
Insecticides

• This is the type of pesticide most likely to cause unintentional neurotoxicity

• Major classes of available insecticides
  • **Organophosphates** and Carbamates
    • OP compounds can be chemical warfare agents.
    • Carbamates include drugs used for treatment of myasthenia gravis and Alzheimer’s Disease.
  • Neonicotinoids
    • Newer agents with recent concerns about effects on pollinating insects.
  • Pyrethrins and Pyrethroids
    • The most commonly available type of insecticide.
  • Phenylpyrazoles and others
OBJECTIVES

• To associate mechanisms of acute organophosphate (OP) toxicity with present and future prospects for treatment.

• To distinguish non-acute toxicities associated with OP exposure, including means for amelioration and prevention.

• To identify potential benefits to medical research resulting from OP compound studies.
Organophosphate and carbamate insecticides are useful but toxic

- These products may be used to protect agricultural crops from insects.
- Toxicities result from poor compliance with label directions, accessible storage or disposal, or inadvertent mix ups or spills.
Neurotoxicity More: OPs and carbamates can cause Acute Signs Due to Excess Acetylcholine (ACh)

- **Signs occur because ACh is not degraded by acetylcholinesterase**
  - Too much neurotransmitter....

- **Signs relate to sites where ACh acts**
  - Central Nervous System (CNS)
  - Neuromuscular junctions
  - Ganglia of the Autonomic Nervous System (ANS)
  - Muscarinic receptors on smooth muscle of peripheral Parasympathetic Nervous System

*Katzung, Basic & Clinical Pharmacol, 12th ed*
• Signs of excess ACh include agonistic effects on Parasympathetic Muscarinic Receptors
  • Atropine blocks these receptors
  • Early administration of oximes may reactivate acetylcholinesterase
• ACh is the neurotransmitter at neuromuscular junctions, causing overstimulation (tremors) and then block
  • Time needed for recovery
• ACh is a transmitter in the brain
  • Available esterase regenerating oximes not effective
  • High dose atropine only marginally effective

Brenner & Stevens, Pharmacology
Neurotoxicity Less: Treatments consider OP mechanisms:

- Atropine to block excess ACh
- Oximes to remove OP from newly inhibited enzyme
- Symptomatic treatment for convulsions
- Decrease exposure
- Time
Neurotoxicity More or Less:
Toxicities of Organophosphates; Treatments

• Acute excess activity in nervous system
  • Accepted treatments: atropine, oximes (2-PAM), diazepam
  • Additional possibilities under investigation.....
    • Exposure prophylactic treatment,
      • but only for threat of OP nerve agent exposures
    • Better anticonvulsants, oximes
    • Administration of esterase enzymes
    • Use of scavengers

• Neuromuscular
  • Muscles no longer contract
  • Time as treatment

• Other effects appearing later.....

Nerve agents
Chemical warfare agents
Cholinesterase inhibitors
Very potent
Volatile

Muscle contraction
Later effects?

Roanoke Times, Mar 20, 1995
Investigations into Decreasing Acute OP Toxicity

**Risk = Hazard + Exposure**
- OPs are hazardous substances
- **Use of protectants and/or scavengers to decrease exposure**
  - *One Example of many possibilities:*
    - derivatized (solubilized) fullerenes
    - These are ‘buckyball’ nanoparticles
    - Advantages include stability and safety

NMR demonstrated a chemical shift of the phosphorus signal, indicative of the sequestering of the OP compound by the fullerene.
Scavengers can protect from OP-induced effects

**in vitro:**

Fullerenes Protect From Paraoxon Induced AChE Inhibition

![Graph showing AChE activity, SH-SY5Y cells, % of control.](image)

**in vivo results:**

Topical application of solubilized fullerenes delayed onset of clinical signs caused by paraoxon that would normally appear in mice ≈ 20 min after exposure.

![Graph showing Mobility percentages.](image)

RISK OF ADVERSE EFFECTS

• Intrinsic hazard
• Dose
• Exposure

• Risk can be reduced by using lower quantities of less hazardous substances, and/or by reducing exposure.
Neurotoxicity More: Other Effects Appearing Later

1. Cognitive / Motor / Psychological
   A. After recovery from serious acute toxicity
   
   B. After long-term, low dose exposure
      1. Epidemiological studies
         • No direct association with esterase inhibition or acetylcholine excess
         • Exposure assessment difficult
         • Variable symptoms
      2. Laboratory studies
         • Developmental? Biochemical?
         • Haven’t been able to reliably reproduce in lab animals clinical effects like those of people
      3. Further research needed

Roanoke Times, Mar 20, 1995
2. Organophosphate-induced *delayed neuropathy*
   - Not possible except with specific OP chemistries
   - Requires early significant and irreversible inhibition of an esterase different from the inhibition of AChE causing acute toxicity (*neuropathy target esterase, NTE*)
   - Progressive peripheral damage to nerve axons doesn’t begin until >7 days after exposure
   - Does not appear in young or in all animal species
   - No treatment
   - Rare, because testing of potential pesticides precludes marketing
     - Some OP compounds have/had industrial uses
   - Modification possible
     - Prophylaxis with reversible inhibitors of NTE
     - Neuroprotective agents decrease clinical and pathological evidence of damage
     - Exacerbated by post-exposure to inhibitors of NTE
Delayed Neuropathy: Damage to nervous system depends on enzyme inhibition, species and time

Neuropathy demonstrated by clinical signs and nerve damage; Potential for damage by NTE inhibition

Timing of effects in susceptible species


Neurotoxicity Less: Nervous System Protectants as Ameliorating agents for OP-induced delayed neuropathy: corticoids and calcium channel blockers

Corticoids are neuroprotective unless dose is too high

Calcium channel blockers ameliorate delayed neuropathy

More or Less: Reversible Neuropathy Target Esterase Inhibitors Protect or Exacerbate

• Pre-exposure to reversible NTE inhibitor decreases availability of enzyme for irreversible inhibition by OP.

• Post-exposure to the same inhibitor worsens neuropathy.

Neurotoxicity Less: Why Marketed Pesticides (OP Insecticides) do not cause delayed neuropathy: Comparison of target esterase inhibitions (AChE for acute effects; NTE for delayed effects)

Esterases differed in sensitivity to OPs.
- Occurred with human and with rodent cells
- AChE much more sensitive than NTE if non-neuropathic

In vitro assays demonstrated 11/11 tested OPs had predictive NTE/AChE inhibitory ratios

RISK OF ADVERSE EFFECTS

• Intrinsic hazard; Dose; Exposure

• Risk can be reduced by using lower quantities of less hazardous substances, and/or by reducing exposure.
  • For OP compounds, Risk is lowered by
    (1) decreasing availability of the most hazard substances
    (2) decreasing exposures with protectants/scavengers
    (3) Prompt general measures to reduce symptoms
    (4) Improved mechanistic interventions
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OPs and potential benefits to medical research

1. Identification of mechanisms associated with neurodegenerative disorders

• Background: OP compounds can be hydrolyzed by esterases that they do not inhibit
  • A-esterases; paraoxonases
    • Paraoxonases have different subtypes; PON2 of interest
  • Work with OP compounds led to the discovery and hypothesis that PON2 is potentially neuroprotective
PON2, an antioxidant enzyme found in female brain > male brain tissue, declines with age


Medical Research: Future prospects for PON2

• Brain antioxidant with sex difference
• Had additive protective effect with estradiol
• Role for PON2 in degenerative diseases?
• Possible reason why males often more susceptible than females to neural aging?

Medical Research (cont.)

2. OP compounds for study of Blood-Brain Barrier (BBB)

- BBB protects brain, but also decreases drug delivery to brain
- Disruption without destruction would be beneficial
- Need to know more about BBB function to investigate


Balbuena et al., Toxicol Sci 114, 260-271, 2010
BBB studies (cont.): OP compounds disrupt BBB

*In vitro* disturbance of BBB is concentration-related

*In vivo* effects time-related; reversible

Balbuena et al., *Toxicol Sci* 114, 260-271, 2010
Learning more about the BBB: OP effect on BBB may be related to effect on transient receptor potential canonical (TRPC) channel subunits

**Effect on TRPC intense but short**

*Balbuena et al., Int J Toxicol 31, 238249, 2011*

*Li & Ehrich, J Appl Toxicol 33, 1187-1191, 2013*
Medical Research: Future prospects for BBB studies

- Mechanisms of disease
  - In vitro / in vivo
- Repair
- Drug delivery
  - Treatment effectiveness

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