Are large animal models helpful for understanding organophosphorus toxicity and for antidote development?

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Funded by

World Health Organisation 2006:
“pesticide poisoning is the single most important global means of suicide”
250-370,000 deaths per year

Cholinergic synapses

Most deaths from OP poisoning occur from respiratory failure

Clinical and public health intervention trials
Animal work & basic science

Reduce global suicide by 10-20% over 10 years (150,000 deaths per yr)

Policy: work with government, WHO, industry
Textbook: OP pesticide poisoning pathophysiology

1. Acute clinical consequences of poisoning are due to inhibition of acetylcholinesterase (AChE) at synapses in autonomic nervous system, NMJ, and CNS
2. Cause of death is respiratory failure
3. Co-formulants are unimportant (cf glyphosate poisoning)
4. Role of co-ingestants unclear

WHO treatment recommendations (Johnson 2000)

- resuscitation & supportive care
- atropine (muscarinic receptor antagonist)
- oximes (to reactivate the inhibited AChE)
- benzodiazepines for sedation
- mechanical ventilation

Paper states “no clinical trials are warranted to test the effectiveness of oximes”

Red cell AChE as marker of responsiveness to oximes

Randomised controlled trial of supportive care plus WHO regimen of pralidoxime or placebo

Pralidoxime pharmacodynamics

Standard testing of pesticide toxicity = rat oral LD₅₀
But the LD50 does not take into account therapy

How well does the rat LD50 reflect human case fatality?

Example of a typical rat study – effect of OP hydrolase

But the animals received no supportive care, no atropine, no ventilation

Why do animal studies?

OP poisoned patients presenting to hospital are heterogeneous. They:
• have ingested varying amounts of different OP pesticides,
• at different times before recruitment, and
• have received different treatments before admission.
Furthermore,
• the dose ingested is rarely known and
• the actual OP ingested may never be known

This great heterogeneity makes clinical research difficult.

All can be resolved with Animal Studies

Requirements for a OP poisoning model

For an animal species to be used for studies addressing the cardiovascular and respiratory systems, and the PK of OP pesticides, certain features will need to be similar to humans:
• absence of carboxylesterase and low levels of AChE in plasma
• human-like foregut allowing similar absorption of pesticides, lavage, and endoscopy
• human-like cardiovascular physiology to permit a similar response to OPs and multi-parametric monitoring using clinical equipment
• human-like lungs to allow bronchoscopy and pathophysiology to be studied
• possible to do studies over many hours or days

How do rodents stand up?

Rodent
• human-like plasma ChE □ No
• human-like foregut □ No
• human-like cardiovascular physiology □ No
• human-like lungs □ No
• long duration studies □ No
And pigs?

<table>
<thead>
<tr>
<th>Rodent</th>
<th>Pig</th>
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Dorandieur 2007. Swine models in the design of more effective medical countermeasures against organophosphorus poisoning. Toxicology 233 (2007) 128

Gottingen minipig model of dimethoate OP poisoning

1. Animal selected due to genetic and environmental homogeneity
2. Studies done under terminal anaesthesia (isoflurane)
3. 15-30 kg male minipigs anaesthetized, ventilated to normocapnia, central arterial/venous lines inserted, attached to LiDCO, Datex and BIS monitors
4. Given 2.5 ml/kg agricultural dimethoate emulsifiable concentrate formulation (EC40) while self-ventilating.
5. Then treated following standard human protocols - fluid resuscitation, atropine, WHO dose oximes, vasopressors, ventilation

Gottingen Minipig

Edinburgh - Translational Medicine

Patients

1. Can we model clinical syndromes of OP poisoning?

Cardiovascular death in dimethoate pesticide poisoning

In poorly resourced hospitals of Asia, it was difficult to study this syndrome in detail
Effects of saline vs dimethoate EC40 2.5 ml/kg

Hyperlactataemia after dimethoate EC40 poisoning

Red cell acetylcholinesterase activity
- OP pharmacodynamics

 paired experiments

Dimethoate EC40 2.5 ml/kg contains:
- 1 g/kg of dimethoate AI (4 rat oral LD50s)
- 1 ml/kg cyclohexanone (0.62 rat oral LD50)
- 0.125 g/kg xylene (0.025 rat oral LD50)
- Wettol surfactant

A. Dimethoate alone - “dimethoate AI”
Prepared as a 25% solution in 40% ethanol (wt Dr Gregorio Naredo)
Dosed with 1 g/kg

B. Cyclohexanone alone
Given as a >99% pure technical solution
Dosed with 1 ml/kg

Arterial blood lactate concentration

Very modest rises in lactate found after both poisonings
Can one reproduce dimethoate EC40 poisoning by combining dimethoate AI with cyclohexanone?

Dimethoate AI 1 g/kg plus Cyclohexanone 1 g/kg

Arterial blood lactate concentration

2. Can we use it to test new antidotes?

Pathophysiology of delayed respiratory failure

Saline placebo Dimethoate EC40 Dimethoate AI
Using nAChRs antagonists to prevent OP-induced NMJ failure

1. Effect of pesticide on NMJ function

2. Protecting NMJ with rocuronium

3. Effect of withdrawing rocuronium

3. Novel models leading to new antidotes

Aspiration pneumonitis – important killer in pesticide poisoning
Model of pesticide aspiration

- Minipigs poisoned with dimethoate EC40 or water
- CT scanned immediately before and then 30 min after gavage
- Then 10 mL of gastric contents placed into one lung.
- Intensive care for next 48 hrs with repeated CT scanning.

CT scanning

Using histogram analysis of CT scans to show changes over time

Can use this 'hard outcome' to study effects of treatments

Overall Conclusions

- Minipig is a useful intensive care model of OP pesticide poisoning, with orders more data collected from each individual animal
- Many similarities to human WHO Class II OP pesticide poisoning
- Used to study the role of solvents in WHO Class II OP toxicity - is this the reason why pralidoxime is not so useful?
- Used to address the role of nicotinic antagonists in preventing neuromuscular junction failure - new antidote?
- Used to develop a model of chemical aspiration with CT imaging that will be used to test new antidotes

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