Using case studies to advance human, animal and regulatory toxicology

Michael Roberts

Clinical Team: Geoff K Isbister, Andrew H Dawson, Nick A Buckley

Analytical Team: Tom Robertson, Ahmed Abdalla, Kushari Burns, Lorraine MacKenzie
Outline

**Our challenge:** Human toxicity includes acute and chronic medicine, chemical poisoning, serious adverse drug reactions and toxicinology.

**Our goal:** Can we characterise such toxicities for the Australian & South East Asian populations, predict such toxicities and/or improve on patient outcomes using regulatory animal and other studies?

**Our resources:** A very productive and well funded NHMRC Program grant that has led to 159 publications in last 5 years, i.e. one every 11 days

**Our scope:** Human toxicology including
- Acute and chronic medicine poisoning
- Chemical poisoning,
- Serious adverse drug reactions and
- Toxinology (e.g. snake and spider bite).
Incidences of poisoning in Australia

- **Data recording** - 60 ICD-10 codes for drug related death in Australia
- **Significance** - accounted for 8.4% of male and 4.8% of female deaths in 2009
- Most deaths are in young people
- **Agrochemical poisoning** - leading cause of death under 65 in rural Asia Pacific

### Deaths and life-years lost from poisoning in Australia in 2009

<table>
<thead>
<tr>
<th>Cause of Death and ICD-10 codes</th>
<th>Persons</th>
<th>Years of potential life lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intentional self-harm (X60-X69)(f)</td>
<td>567</td>
<td>17, 161</td>
</tr>
<tr>
<td>Accidental poisoning by/ exposure to noxious substances (X40-X49)</td>
<td>799</td>
<td>28,553</td>
</tr>
<tr>
<td>Event of undetermined intent (Y10-Y34)</td>
<td>367</td>
<td>11,597</td>
</tr>
<tr>
<td>Total</td>
<td>1733</td>
<td>57,311</td>
</tr>
</tbody>
</table>
Desirable outcomes

**Overall:** To translate human toxicology knowledge using clinical, epidemiological, cell and mechanistic perspectives

**Specific:**
- Improved detection of emerging toxicological issues in humans (toxicovigilance)
- Understanding toxicological mechanisms behind differential toxicity in humans
- Improved management of clinical toxicological problems
- Providing clinical toxicology data to facilitate public-health and regulatory responses
Traditional regulatory approach to define safe & effective drug disposition

Phase 4 post-market surveillance

Phase 3 studies in broader population (1000 - 3000)

Plan

Phase 2 studies in targeted condition (100 – 300)

Verify Simulations

Phase I volunteer PK studies (20 – 100)

Micro-dosing in man?

Simulate

Compound properties

In silico, In vitro, animal data

Verify Simulations

Bottom up physiological (mechanistic) pharmacokinetic approach
But safety to chemicals is more complex as can’t do the same studies in man as for drugs!

Adapted from Goldsmith et al 2012
Can we treat poisoning better? Can we use our clinical data to improve regulatory toxicology?

One way to better understand the potential safety issues of chemicals in humans is to measure the responses and blood levels after poisoning and distil that down – a top down approach!

- We, (Dawson et al 2010) examined a series of 9302 human pesticide poisonings & found an overall case-fatality of around 10%

- Our challenge is that we rarely know what dose our poisoned patients are exposed but much of the animal safety data is in doses
- Need blood data & measure same pathology/biomarkers in both animals and man so we can compare “apples with apples”
Our case study approach!

- A poisoned patient arrives at an Emergency Department in a hospital somewhere in Australia or in South East Asia (Sri Lanka)
- Patient’s clinical condition, pathology recorded, treatment initiated and a series of timed blood samples are taken
- We then measure drug & antidote concentrations in plasma, derive the clinical pharmacokinetics & relate to pathology changes
Paraquat – my introduction to quantitative clinical toxicology

*Kinetics of toxic doses of paraquat and the effects of hemoperfusion in the dog*  

- Complex pharmacokinetics as the herbicide, paraquat-induced renal failure leading to dose- and time-dependent elimination
- Haemoperfusion only made a difference to deep tissue levels if infused within 2 hrs of ingestion
- More recently explored animal and human toxicity in more detail

Population analysis of paraquat toxicokinetics in poisoning patients  

Australia, Thailand, Sri Lanka
Renal biomarkers predict nephrotoxicity after paraquat

Klintean Wunnapuk, Xin Liu, Philip Peake, Glenda Gobe, Zoltan Endre, Jeffrey E. Grice, Michael S. Roberts, Nicholas A. Buckley

- Seven renal injury biomarkers used to detect early renal damage and dysfunction and to compare with the conventional endogenous marker creatinine
- Male Wistar rats dosed with paraquat, and the biomarker patterns in urine and plasma were investigated at 8, 24 and 48 h
- Urinary kidney injury molecule-1 was the best marker at predicting histological changes as early as 8 h
- Urinary kidney injury molecule-1, urinary albumin and urinary cystatin-C elevations correlated with the degree of renal damage and injury development
- Excretion rate and normalised biomarker concentration did not improve biomarkers diagnosis in predicting histological changes
- Plasma cystatin-C mirrored renal function as well as plasma creatinine

![Time-course of changes in injury biomarker concentrations. Kidney injury molecule-1 (A), albumin (B), cystatin-C (C and D), neutrophil gelatinase-associated lipocalin(E) and creatinine (F)]
Some case examples – isoniazid poisoning

Isoniazid, synthetic derivative of nicotinamide (vitamin B3), is “an antibiotic used as a first-line agent for the prevention and treatment of both latent and active tuberculosis”. Mycobacteria, particularly Mycobacterium tuberculosis (and) some atypical types of mycobacteria, such as M. kansasii and M. xenopi.

Wikipedia
What do we know about isoniazid pharmacokinetics & toxicity?

Single-dose concentration-versus-time PK profiles for incremental oral doses of isoniazid in uninfected BALB/c mice (mean ± SEM)

Linear pharmacokinetics with terminal elimination half-life 0.4 to 1.6 h

Relationship between AUC$_{24}$/MIC (A) & $C_{\text{max}}$/MIC (B), and and log$_{10}$CFU/lung of *M. tuberculosis* (mean ± SEM) when the total dose as 6, 12, or 18 equally divided doses in 144 h

Isoniazid (INH) toxicity

• Acute overdose of INH can cause sudden onset of seizures and “slow acetylators” more readily develop side effects on chronic dosing e.g. peripheral neuropathy, agitation, insomnia, and abdominal complaints

• Acidemia from INH-induced seizures profound

• Therapy involves:
  • Management of airway, breathing, and circulation
  • Pyridoxine antidote (vitamin B6) given gram-per-gram to stop seizures and improve acidosis
  • Supportive care
  • Used for patients with oral dose < 20 mg/kg (1.4 g/70 kg) and asymptomatic for 4 hours

• Animals vary in response:
  • Safe use in many species
  • Low (LD50 50 mg/kg) and narrow margin of safety in dogs (poor acetylators) of isoniazid
**Case study**

**History:**
- 20-yr-old woman/recurrent seizures following ingesting INH (25 g)

**Treatment:**
- Activated charcoal
- Midazolam
- Pyridoxine (14 mg)
- High-volume continuous venovenous hemodiafiltration (CVVHDF)
- Full recovery in 4 days

**Methods:**
- Five plasma samples over 40 hrs
- INH quantified (LC-MS)
- A pharmacokinetic analysis: two compartment model

**Results:**
- Initially good clearance with CVVHDF (4 times endogenous clearance) which rapidly declined within hours
Levetiracetam

• Levetiracetam (S-enantiomer of etiracetam) is used to treat epilepsy but its exact mechanism is unknown and it can increase the risk of suicide behavior or thoughts

• An anticonvulsant used to control canine epilepsy

• **Overdose** symptoms may include extreme drowsiness, agitation, aggression, shallow
Comparative pharmacokinetics & toxicity of Levetiracetam

• Pharmacokinetics of levetiracetam are linear over the dose range of 500-5,000 mg in man

• In humans, <10% protein bound and ~66 percent excreted unchanged into urine. Adult plasma half-life is about 6 to 8 hours

• Predominant route of elimination of total $^{14}$C levetiracetam was in urine, accounting for 81, 93, 87 and 89% of the dose in the mouse, rat, rabbit and dog. 

Xenobiotica. 2004 Mar;34(3):281-300

• Toxicity profile after single and repeat i.v. doses in mice, rats and dogs
  • Minimal clinical signs seen for up to 1,800 mg/kg/day in rats or 1,200 mg/kg/day in dogs
  • Most common are neuromuscular effects and salivation and, in dogs, emesis
Cardiovascular toxicity with levetiracetam overdose

Colin B. Page, Ahmed Mostafa, Ana Saiaco, Jeffrey E. Grice, Michael S. Roberts and Geoffrey K. Isbister

- **Case study**

- **History:**
  - 43-yr-old female/mild CNS depression, bradycardia, hypotension and oliguria post ingestion of 60–80 g of levetiracetam

- **Treatment:**
  - Atropine and intravenous fluids
  - Full recovery in 48 hrs

- **Methods / Results:**
  - Levetiracetam concentration: 463 mcg/ml (therapeutic range: 10–40 mcg/ml)
  - Concentration–time data fitted to one compartment model with first-order input
  - Elimination half-life: 10.4 hrs

(A) Serum levetiracetam concentrations with modeled time concentration curve for 70 g
(B) Patient’s reported range of ingested dose of 60 & 80g
MCPA (2-methyl-4-chlorophenoxyacetic acid) and Bromoxynil

- MCPA: a powerful, selective, widely used phenoxy herbicide, selective for plants with broad leaves
- Bromoxynil: a nitrile herbicide also used for post-emergent control of annual broadleaf weeds

BRIEF COMMUNICATION

2-Methyl-4-chlorophenoxyacetic acid and bromoxynil herbicide death

INGRID BERLING,1,2 NICHOLAS A. BUCKLEY,3 AHMED MOSTAFA,4,5 MICHAEL A. DOWNES,1,2 JEFFREY GRICE,4 GREGORY MEDLEY,4 MICHAEL S. ROBERTS,4,6 & GEOFFREY K. ISBISTER1,2

1School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia
2Department of Clinical Toxicology and Pharmacology, Calvary Mater Newcastle, Newcastle, NSW, Australia
3Clinical Pharmacology, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia
4Therapeutics Research Centre, University of Queensland, Brisbane, Australia
5Pharmaceutical Chemistry Department, Faculty of Pharmacy, Helwan University, Egypt
6School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia
MCPA

• Field half-life of 14 days to one month, and has a low affinity for soil

• Controls a wide range of broadleaf weeds by accumulating in the meristematic tissue where it stimulates plant hormones disrupting both new seedling and existing plant growth

• Low toxicity in mammals, oral LD50 765 mg/kg; dermal LD50 exceeds 2,000 mg/kg, and the inhalation LC50 exceeds 6.3 mg/L in rats (#EPA)

• Human lethal oral dose 250 - 450 mg/kg (estimated)

• Note non-linear (saturable) pharmacokinetics at high plasma levels associated with poisoning

Bromoxynil

- Bromoxynil: a nitrile herbicide that inhibits photosynthesis of post-emergent control of annual broadleaved weeds
- Half-life of bromoxynil is about ten days in sandy soil where broken down to less toxic compounds
- Reported oral LD50s of 190 mg/kg in rats, 260 mg/kg in rabbits, and 63 mg/kg in guinea pigs
- Highly toxic to pheasants (LC50 of 50 mg/kg) and is moderately toxic to hens (LC50 of 240 mg/kg), quail (LC50 of 100 mg/kg), and mallard ducks (LC50 of 200 mg/kg)
- **Species differences?** Dogs fed bromoxynil for 90 days showed unspecified adverse effects at and above 5 mg/kg but no observable effect on rats at or below 16.6 mg/kg/day
2-Methyl-4-chlorophenoxyacetic acid and bromoxynil herbicide death

Ingrid Berling, Nicholas A. Buckley, Ahmed Mostafa, Michael A. Downes, Jeffrey Grice, Gregory Medley, Michael S. Roberts and Geoffrey K. Isbister

❑ Case Study

❑ History:
  • 37-yr-old man/nausea, vomiting and diarrhoea
  • Ingestion of MCPA/bromoxynil co-formulation herbicide
  • Clinical assessment prior to death:
    • increased CO₂ production, hyperthermia, metabolic derangement, cardiac asystole and death

❑ Methods /Results:
  • MCPA 84 mg/mL and bromoxynil 137 mg/mL
  • quantified by LCMS

❑ Discussion:
  • Mechanism of death: uncoupling of oxidative phosphorylation, excess CO₂ production, hyperthermia
  • Limited knowledge on the acute toxicity of herbicides - bromoxynil

Trends of RR, CO₂, pH, temperature and lactate level from presentation to death
Aldicarb

- Carbamate insecticide - acetylcholine cholinesterase inhibitor
- Severe poisoning leads to death by respiratory failure
- Classified as a Restricted Use Pesticide (RUP) in the United States
- Illegal use of aldicarb to maliciously poison dogs is a major problem in some parts of the world – major cause of dog poisoning in South Africa,
- Seven farm workers shared a watermelon and presented to a rural emergency department with symptoms of cholinergic poisoning. They were treated empirically with atropine and pralidoxime. Analyst verified aldicarb in the watermelon samples. *J Agromedicine. 2013;18(2):174-7*
- EPA - Bayer CropScience agreed to stop producing aldicarb by 2015 in all world markets after aldicarb poisoning sickened more than 2,000 people who had eaten California watermelons.
The pharmacokinetics and pharmacodynamics of severe aldicarb toxicity after overdose

Adam P. Michael, Ahmed Mostafa, Joyce M. Cooper, Jeffrey Grice, Michael S. Roberts & Geoffrey K. Isbister

**Case Study**

**History:**
- 57-yr-old female/unconscious-ingestion of aldicarb
- Symptoms: bradycardia, hypotension, hypersalivation, clammy, small pupils, and weakness

**Results:**
- Aldicarb concentration: 2.18 μg/ml
- Pharmacokinetic analysis:
  - two compartmental model
  - first order absorption, time of ingestion 4.5 h pre-admission
  - half-life of distribution 0.4 h and half-life of elimination, 13 h
- Cholinesterase concentration and IC$_{50}$:
  - Plasma Conc: 0.3 kU/L (RR: 4.3 – 10.6 kU/L), IC$_{50}$: 0.15 μg/ml
  - Red Cell Conc: 10 U/gHb (RR: 38 – 66 U/gHb), IC$_{50}$: 0.26 μg/ml

**Discussion:**
- Aldicarb poisoning causes rapid onset severe toxicity
- Cholinesterases rapidly recover once aldicarb concentrations decrease and precede clinical recovery

Plots of observed measurements of the plasma, red cell cholinesterase and plasma aldicarb concentrations
Midodrine

- Vasopressor / antihypotensive agent
- Used in treatment for low blood pressure
- Main (active) metabolite desglymidodrine formed by deglycination
- Desglymidodrine (alpha-agonist) - activates alpha-adrenergic receptors of the arteriolar and venous vasculature
- This results in an increase in vascular tone and elevation of blood pressure
- Half-life: midodrine ~ 30min, desglymidodrine 3-4 h
Severe Hypertension and Bradycardia Secondary to Midodrine Overdose

Lee Y. Wong, Anselm Wong, Thomas A. Robertson, Kushari Burns, Michael S. Roberts and Geoffrey K. Isbister (2016)

Case study

History:
- 22-yr-old female/severe hypotension, vomiting, bradycardia
- Taken 350mg midodrine
- Treated with vasodilator agents/supportive care
- Discharged on day 3

Methods/Results:
- Plasma midodrine and desglymidodrine concentrations measured with LCMS
- 2 h post-ingestion concentrations of parent drug and metabolite 158 and 170 ng/mL
- Parent drug concentrations rapidly decreased (elimination half life 1.6h)
Metoprolol

- β1-adrenergic antagonist (beta-blocker)
- Treatment for acute myocardial infarction, heart failure, angina and hypertension
- Improves blood flow & lowers blood pressure by relaxing blood vessels & slowing heart rate
- Extensively metabolised by CYP2D6
- Half-life 3-4h (except poor metabolisers)
- (Fatal / non-fatal overdose cases reported; Stajic et al 1989)
Zero-order metoprolol pharmacokinetics after therapeutic doses: severe toxicity and cardiogenic shock

Geoffrey K. Isbister, Karyn Ang, Kieron Gorman, Joyce Cooper, Ahmed Mostafa and Michael S. Roberts

❖ **Case study**

❖ **History:**
  - 90-yr-old female/hypotension, cardiogenic shock, multi-organ failure
  - Post metoprolol dose of 250 mg/day

❖ **Treatment:**
  - Fluid resuscitation, vasopressor support, insulin infusion
  - Continued to improve 36 h post admission

❖ **Methods/Results:**
  - Metoprolol concentration: 2.39 µg/mL (therapeutic range 0.035–0.5 µg/mL)
  - Concentration–time data fitted to one compartment model with Michaelis–Menten (MM) kinetics and zero order elimination
    - $V = 63.4 \text{ L}$, $V_{\text{max}} = 9.57 \text{ mg/h}$, $K_m = 0.97 \text{ mg/L}$
    - Elimination half-life: 9 h (decreased from 20 h)
    - Prolonged toxicity possibly - poor CYP2D6 metabolism
High lethality and minimal variation after acute self-poisoning with carbamate insecticides in Sri Lanka – implications for global suicide prevention

Thomas Lamb\textsuperscript{a}, Liza R. Selvarajah\textsuperscript{a}, Fahim Mohamed\textsuperscript{b,c}, Shaluka Jayamanna\textsuperscript{b,d}, Indika Gawarammana\textsuperscript{b}, Ahmed Mostafa\textsuperscript{e,f}, Nicholas A. Buckley\textsuperscript{b,c}, Michael S. Roberts\textsuperscript{e} and Michael Eddleston\textsuperscript{a,b}

United Kingdom\textsuperscript{a}, Sri Lanka\textsuperscript{b,d}, Australia\textsuperscript{c,e} and Egypt\textsuperscript{f}

Carbosulfan

Fenobucarb

Carbofuran
Pesticide poisoning

❖ Background:
  • Pesticide self-poisoning - major health problem world wide
  • Carbamates used as alternative to more toxic insecticides containing organophosphates (OP)
  • Carbamates inhibit AChE resulting in respiratory failure

❖ Methods:
  • Studied 1288 patients self-poisoned with carbamates
  • Plasma carbamate concentrations measured by LCMS

❖ Results:
  • Median plasma carbamates concentrations were carbofuran, 54 µg/L (n=719), carbosulfan 18 µg/L (n=389), and fenobucarb 1160 µg/L (n=127)
  • No significant variation of self-poisoning found according to type of carbamate ingested
  • Carbamates did not appear to be less toxic than OP in human poisoning

Time between ingestion and death for patients poisoned by the three carbamates. Bars show the median(IQR) time
Pesticide poisoning
How well does rat LD50 correlate with observed human toxicity?

Median human plasma carbamates concentrations/time of death
- carbofuran, 54 µg/L/42.3h = 1.28 µg/L/h
- carbosulfan 18 µg/L/21.3h = 0.84 µg/L/h
- fenobucarb 1160 µg/L/25.3 h = 45.85 µg/L/h

Median human plasma carbamates concentrations/case fatality (% died)
- carbofuran, 54 µg/L/2.2 = 24.5 µg/L
- carbosulfan 18 µg/L/11.1 = 1.6 µg/L
- fenobucarb 1160 µg/L/6.3 = 184 µg/L

Carbosulfan < Carbofuran < Fenobucarb

Effective concentration for a time to death
- Carbofuran 8.08 µg/L/h
- Carbosulfan 1.6 µg/L/h
- Fenobucarb 45.85 µg/L/h

Effective fatal concentration (µg/L)
- Carbofuran < Carbosulfan < Fenobucarb

LD50 (mg/kg): Carbofuran 8.08 < Carbosulfan 269 < Fenobucarb 626
Managing chloroform overdose – does nature of antidote regimen matter?

Our case:
- 39 year old female swallowed about 20-30 mL chloroform.
- Intubated and intravenous acetylcysteine given as an infusion from 42 to 72 h at 6.25 mg/kg/hr.

Literature case:
- 19-year-old African-American man swallowed about 75 mL of chloroform. – immediate intubation and mechanical ventilation with serum chloroform concentration on admission was 45 times our peak level. = 91 μg/mL.
- Immediately infused with N-acetylcysteine 150 mg/kg over 1 h, then 50 mg/kg over 4 h, and finally intravenous drip at 6.25 mg kg⁻¹ h⁻¹ and stopped on day 6

Managing chloroform overdose
Choosing treatment regimens

Implication 1: In an overdose, consider starting NAC early & high, then tailor down!!
- 75 mL chloroform → 91 μg/mL peak – rapid/high NAC, then tailor down → ALT peak 510 U/L
- 20-30 mL chloroform → 2 μg/mL peak – start low dose NAC after 42 hr → ALT peak 1200 U/L

Implication 2: Consider other treatments

Mouse hepatocyte data chloroform-induced cell death in 2 phases: shows that
- a metabolic phase with glutathione depletion
- an oxidative phase with lethal mitochondrial permeability transition (MPT) and protein nitration

Treatment: MPT inhibitor – CsA, antioxidant NAC & others
- CsA blocked oxidative stress
- Loss of mitochondrial membrane potential is blocked by CsA or NAC
- Protein nitration blocked by CsA or NAC

Implication 3: Learn from other poisonings

Paracetamol is also a mitochondrial poison: It forms reactive NAPQI that is detoxified at therapeutic doses by glutathione. We now paracetamol toxicity also causes MPT.
Targeting mitochondria with methylene blue protects mice against acetaminophen-induced liver injury

Pohl et al Biochem Biophys Res Commun 79 (3) 684-691, 1977
Lee et al Hepatol 61:326-336, 2014
A global increase in the availability and use of novel psychoactive substances (NPS) over the last decade—one example is phenibut (β-phenyl-γ-aminobutyric acid), a GABA<sub>B</sub> agonist. According to the grey literature, phenibut is taken for its anxiolytic and euphoric properties, with tolerance and withdrawal syndromes commonly reported adverse effects. Phenibut oral average dose 2.4 g.
Acute behavioural disturbance associated with phenibut purchased via an internet supplier

**Context: Toxicity from recreational substances**

*Case Study 1*
- 20-yr-old female/decreased level of consciousness, delirium
- Having taken phenibut the prior day
- Given supportive care/toxicology service
- Made a full recovery over a 24-hour period
- Admitted to use of phenibut purchased online for recreational purposes
- Plasma phenibut concentration 29.7 µg/ml (analysed by LCMS)

*Case Study 2*
- A 38-yr-old male/agitated delirium
- Having used tetrahydrocannabinol, alcohol and phenibut, prior evening
- Subsequently intubated for airway protection / ongoing sedation
- Admitted to using phenibut purchased online
- Plasma phenibut concentration 36.5 µg/ml (analysed by LCMS)
Some concluding thoughts!
Comparative animal and human studies – allometry, physiological pharmacokinetic & margin of safety (MoS) approaches

- Algometric relations and scaling laws-relatively simple power-low relationship with body weight
- Physiological pharmacokinetic approaches based on complex set of physiological processes and biochemical interactions
- Need to move away from LD50 to NOAEL and LOAEL
- Need to accurate define MoS rather than simply have it as an empirical safety factor that has a safe human dose as being 1/1000<sup>th</sup> or 1/100<sup>th</sup> the NOAEL for a chemical in animals

\[ Y = a BW^b \]

Y=anatomic size, a=empirical coefficient, BW=body weight, b=allometric exponent

Generic PPBPK model of the mammalian body.
IN CONCLUSION

Whatever you can do or dream you can, begin it.
Boldness has genius, power and magic in it!

Thank you also to my team, present & former students, granting bodies (NHMRC, ARC, FDA) for their support and you for listening!

Recognising that, sometimes, paradigms should be challenged

Goethe

Therapeutics Research Centre: Adelaide Team