Biokinetic considerations in the use of in vitro systems for estimating acute (systemic) toxicity

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The new paradigm in toxicology

toxicity is determined by:
the critical concentration and time of exposure (dose metric) to
the critical compound (metabolite?) at
the critical site of action
use of in vitro cytotoxicity data in estimating in vivo acute toxicity

• Assumptions:
• basal cytotoxicity (EC50) is a good predictor for acute toxicity in vivo
• cytotoxic concentrations in vitro mirror blood plasma concentrations
• blood plasma concentrations mirror target tissue concentrations
• but….there are complicating factors
Local vs systemic effects

• If a compound would quickly act on each and every cell type, then the effects would be the most obvious on the place of application (skin, lungs, gi tract: oesophagus, stomach): local effects.

• Systemic toxicity will appear if the process of toxicity is less fast or if other cell types are more sensitive/less well protected.
reasons for deviations

• quality of in vivo and/or in vitro data

• cytotoxic concentration is irrelevant for in vivo target tissue concentration
relevance of in vitro concentrations (1)

• specific organ or tissue toxicity

main organs relevant for acute toxicity: brain, liver, kidney, lung (local ?)
relevance of in vitro concentrations (2)

• biokinetic behaviour of the compound in organism:

• 1. absorption: if limited: in vitro concentrations will be higher than in vivo plasma concentrations: overestimation of toxicity
relevance of in vitro concentrations (3)

• *biokinetic* behaviour of the compound in organism:

• 2. *distribution* of compound leads to higher or lower concentrations on target site

• example: fat tissue, CNS
relevance of in vitro concentrations (4)

- **biokinetic** behaviour of the compound in organism:

3. **elimination:**
- fast excretion/exhalation
- fast metabolism:
  - fast elimination or formation of toxic metabolite
relevance of in vitro concentrations (5)

• quality of in vitro data:
• “biokinetics in vitro”
• protein binding, medium vs cells, binding to plastic, evaporation
• nominal vs real free concentration
Cytotoxicity vs Serum Level

![Graph showing cytotoxicity vs serum level]

### Table: Measured % Free NCS

<table>
<thead>
<tr>
<th>% NCS</th>
<th>% Free</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>6.85 ± 0.75</td>
</tr>
<tr>
<td>2.5</td>
<td>4.55 ± 1.01</td>
</tr>
<tr>
<td>5</td>
<td>2.70 ± 0.20</td>
</tr>
</tbody>
</table>

### Table: EC₅₀ (µM)

<table>
<thead>
<tr>
<th>NCS (%)</th>
<th>Log EC₅₀ (µM)</th>
<th>EC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>1.79 ± 0.06</td>
<td>61.3</td>
</tr>
<tr>
<td>2.5</td>
<td>2.02 ± 0.04</td>
<td>2.15</td>
</tr>
<tr>
<td>5</td>
<td>2.22 ± 0.03</td>
<td>104.1</td>
</tr>
</tbody>
</table>

**Legend:**
- Free
- Nominal
- 1.25% NCS
- 2.5% NCS
- 5% NCS

**Sealer:**
- Well
- Fiber
- Medium
- Cells
Modeling Free Concentration

\[ F = \frac{1}{1 + K_S[S] + K_P[P] + K_C[C] + K_a \cdot \frac{V_a}{V_m}} \]

- Serum protein binding
Modeling Free Concentration

\[
F = \frac{1}{1 + K_s[S] + K_p[P] + K_c[C] + K_a \cdot \frac{V_a}{V_m}}
\]

- Plastic binding
biokinetics in ACuteTox

• prediction of biokinetics from PhysChem
• in vitro absorption
• in vitro blood-brain barrier
• PBBK modelling

• collect data for prediction of alerts and correctors
biokinetics in ACuteTox

- find **alerts** and **correctors** for improving interpretation of basal cytotoxicity data

- incorporate these in logical and transparent strategy
$Ka = 0.3, 1 \text{ and } 3; CLh_{int} = 10, CLk_{int} = 10$
Ka = 1; Various CL\textsubscript{k\_int}

C\text{max plasma in liver vene}

C\text{max pooled plasma}

log(Kow)
NOW: how to make this practical
Toolboxes

1. Pre-existing data
2. Physico-chemical properties (theoretical)
3. Simple test battery using cell lines
4. PBPK modelling
5. Target-specific toxicity
6. Verify acute toxicity
First step:

• When is basal cytotoxicity sufficient:
  • When the compound is rapidly absorbed;
  • When no aberrant distribution pattern is encountered
  • When no fast (metabolic) clearance occurs
  • When acute tox is not the result of something else than basal cytotox: specific target tox.
  • When in vitro freely available concentrations do not deviate much from nominal concentrations
Next step:

- When do we decide that the criteria for inclusion in the “ideal” algorithm are NOT fulfilled?
- i.e. when does absorption become a reason for deviation?
- Can we decide on the basis of phys-chem properties
  - in vitro data
Same exercise for:

- Distribution
- Metabolism
- Excretion
- Neurotox
- Hepatotox
- Nephrotox
- Hematotox
- In vitro biokinetics
**EC50 vs LD50:** 0.3445

**estLD50 ign kinetics vs LD50:** 0.3457

**estLD50 incl kinetics vs LD50:** 0.5396
Conclusion

1. Need to take in vitro biokinetics into consideration: will improve quality of in vitro toxicity data
2. Use kinetic parameters to correlate in vitro effective concentrations to a dose in vitro: QIVIVE.
3. Use approach in improving the applicability of in vitro data in risk assessment
Acknowledgement

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