INTEGRATING BIOKINETICS AND BIODYNAMICS FOR CONSUMER SAFETY ASSESSMENT USING QUERCETIN AS A CASE STUDY

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OVERVIEW

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  » TT21C
  » DNA damage project

• Case study
  » Approach
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  » Biodynamics
  » *In vitro to in vivo* extrapolation and risk assessment
  » Building confidence in
  » Summary and conclusion
“Advances in toxicogenomics, bioinformatics, systems biology and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biological processes using cells of human origin.”

- Case study approach used to implement TT21C vision
INTRODUCTION: DNA DAMAGE PROJECT

Traditionally “risk assessment” of ‘genotoxins’ have been based on linear models.

Our aim is to understand how safety may be assured for complex toxicological endpoints using data derived from a toxicity pathways-based approach that is rooted in mechanistic understanding of the underlying biology.
CASE STUDY: QUERCETIN

- Flavonoid present in green tea, berries and vegetables
- Positive results in some *in vitro* genotoxicity tests, but is considered not to be a carcinogen (Harwood et al 2007)
- Question: Can we perform a hypothetical risk assessment based on perturbation of the p53 pathway for 0.5% quercetin in a skin lotion (systemic exposure)

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Adeleye et al 2015
APPROACH

Exposure Scenario (0.5% Quercetin in skin lotion)

- Define rate of exposure through skin (µg/cm²/hr)
  - Exposed area = Amt. of product applied * Exposed area
  - Exposed area * infusion rate * time
  - PBPK model (fitted to in vivo data)

- Define amount entering blood (mg)
- Define plasma concentration at steady state (Cmax µg/ml)
- Reverse Dosimetry to determine relationship between plasma concentration and external dose

- Chemistry (Do you need to measure free concentration?)

- Define Nominal BPAD (µM)
- Adjust BPAD Time weighted average (µM)

- Establish in vitro free concentration of chemical (µM)
- Establish total chemical plasma concentration at steady state (µg/ml)

- External dose required to produce internal effect (µg/cm²)
  - 100 * External dose / (density * volume)

- Percent in Product

- Micronucleus, DNA repair centre assay, Transcriptomics
  - Time concentration profile, AUC/time
  - Chemical binding to serum (rapid equilibrium dialysis)
    - TWA BPAD * percent free
    - 2.6µM * 0.359
  - Free TWA BPAD ÷ Fraction unbound in vivo (literature or PBPK model)
    - 0.28µg/ml ÷ 0.02
APPROACH: BIOKINETICS

1. Define rate of exposure through skin (μg/cm²/hr)
   - Concentration in formulation, skin penetration, skin model

2. Define exposed area
   - Exposed area = Amt. of product applied * Exposed area

3. Define amount entering blood (mg)
   - Exposed area * infusion rate * time

4. Define plasma concentration at steady state (Cmax μg/ml)
   - PBPK model (fitted to in vivo data)

5. Reverse dosimetry to determine relationship between plasma concentration and external dose

Define amount entering blood

- Assume that 5 mg/cm² of lotion is required (SCCS 2010).
- Amount of Body Lotion applied:
  - Worst Case: 14.39 g = 2,878 cm².
  - Median: 7.63 g = 1,526 cm².
- Exposed area = amount of product applied * area of skin applied
- Amount in blood = Exposed area * infusion rate
**APPROACH: BIOKINETICS**

- Define plasma concentration at steady state
- Reverse dosimetry to determine relationship between plasma concentration and external dose
- PBPK model fitted to *in vivo* data
- Apply scenario (repeat dose every 24hrs)
- Simulate until steady state achieved
- Run for both scenarios

- Run Gastro Plus® to set a value for the concentration of chemical in plasma and optimise on dose
- Model will provide the “surface dose” that produces the specified concentration.
- The relationship between the dose on the surface of the skin (μg/cm²) predicted by the plasma concentration at steady state (μg/ml) is linear
**APPROACH: BIODYNAMICS**

Exposure Scenario (0.5% Quercetin in skin lotion)

- **Define rate of exposure through skin (\(\mu g/cm^2/hr\))**
- **Define amount entering blood (mg)**
- **Define plasma concentration at steady state (Cmax \(\mu g/ml\))**
- **Reverse Dosimetry to determine relationship between plasma concentration and external dose**
- **Chemistry (Do you need to measure free concentration?)**
- **Define Nominal BPAD (\(\mu M\))**
- **Adjust BPAD Time weighted average (\(\mu M\))**
- **Establish in vitro free concentration of chemical (\(\mu M\))**
- **Establish total chemical plasma concentration at steady state (\(\mu g/ml\))**
- **External dose required to produce internal effect (\(\mu g/cm^2\))**
- **Percent in Product**

- Concentration in formulation, Skin penetration, Skin model
- Exposed area = Amt. of product applied * Exposed area
- Exposed area * infusion rate * time
- PBPK model (fitted to in vivo data)
- Optimise on dose; time and surface area (PBPK)
- Micronucleus, DNA repair centre assay, Transcriptomics
- Time concentration profile, AUC/time
- Chemical binding to serum (rapid equilibrium dialysis) TWA BPAD * percent free 2.6\(\mu M\) * 0.359
- Free TWA BPAD / Fraction unbound in vivo (literature or PBPK model) 0.28\(\mu g/ml\) / 0.02
Homeostasis likely requires perfect adaptation of both rapidly acting pathways (post-translational modification) and slower acting pathways (transcriptional).

Lower doses - rapid post translational modification

Higher doses: At some point (depletion of p53 reserves or other post-translational modification), pathway moves to transcriptional control.

Approach: Biodynamics

Repair centre quantification

Making a Safety Assessment decision on the Adaption to Adversity ‘Tipping Point’

A POD or BPAD but accompanied by mechanistic rationale

Diagram from Rebecca Clewell

- Homeostasis likely requires perfect adaptation of both rapidly acting pathways (post-translational modification) and slower acting pathways (transcriptional).
- Lower doses: rapid post translational modification
- Higher doses: At some point (depletion of p53 reserves or other post-translational modification), pathway moves to transcriptional control
APPROACH: BIODYNAMICS BPAD DEFINITION

- Based on the DNA repair centre results, MN assay and transcriptomics we determined the BPAD as the highest dose where
  - No MN is observed DNA repair centres are resolved
  - No transcriptional changes are observed

BPAD for Quercetin is 10µM
APPROACH: **IN VITRO KINETICS**

**Exposure Scenario (0.5% Quercetin in skin lotion)**

- **Define rate of exposure through skin (μg/cm²/hr)**
- **Define amount entering blood (mg)**
- **Define plasma concentration at steady state (Cmax μg/ml)**
- **Reverse Dosimetry to determine relationship between plasma concentration and external dose**

**Chemistry (Do you need to measure free concentration?)**

- **Define Nominal BPAD (μM)**
- **Adjust BPAD**
  - Time weighted average (μM)

- **Establish in vitro free concentration of chemical (μM)**

- **Establish total chemical plasma concentration at steady state (μg/ml)**

- **External dose required to produce internal effect (μg/cm²)**
  - $100 \times \frac{\text{External dose}}{(\text{density} \times \text{volume})}$

**Percent in Product**

- Micronucleus, DNA repair centre assay, Transcriptomics
- Time concentration profile, AUC/time
- Chemical binding to serum (rapid equilibrium dialysis)
- TWA BPAD * percent free
- $2.6 \mu M \times 0.359$
- Free TWA BPAD ÷ Fraction unbound in vivo (literature or PBPK model)
- $0.28 \mu M ÷ 0.02$

**Conc. in formulation, Skin penetration, Skin model**

- Exposed area= Amt. of product applied* Exposed area
- Exposed area*infusion rate*time
- PBPK model (fitted to in vivo data)
- Optimise on dose; time and surface area (PBPK)
APPROACH: *IN VITRO KINETICS*

Quercetin is unstable and degrades over time.
- Time concentration profiles were generated.
- Area under the curve was calculated.
- The time weighted average (TWA) BPAD for Quercetin was 2.6 µM (0.79 µg/ml).
**APPROACH: FREE CONCENTRATION OF QUERCETIN**

- Binding of quercetin to foetal calf serum was assessed in 5% foetal calf serum at eight concentrations ranging from 2.5 to 125 \( \mu g/ml \) using Rapid Equilibrium Dialysis.

- Different binding equations can be fitted based on different assumptions about the mechanism of binding of the test chemical to BSA. (AIC was used to select the best model)
  
  - 1 single saturable binding site
  - Two saturable binding sites
  - Single binding site (saturable) plus non-specific (non-saturable) binding

- \(~67.7\%\) quercetin is bound and \(32.3\%\) free
Reverse Dosimetry: same freely available concentration in vitro and in vivo will result in the same magnitude of effect.

- Free TWA BPAD concentration in vitro equals the steady state free plasma BPAD concentration in vivo.
- Total plasma steady state concentration = free conc. /fraction unbound in vivo
  - 0.26/0.02
  - 13 μg/ml
  - Value used as input in PBPK model
QUERCETIN RISK ASSESSMENT

Exposure Scenario (0.5% Quercetin in skin lotion)

- Define rate of exposure through skin ($\mu$g/cm²/hr)
- Define amount entering blood (mg)
- Define plasma concentration at steady state (Cmax $\mu$g/ml)
- Reverse Dosimetry to determine relationship between plasma concentration and external dose
- Define Nominal BPAD ($\mu$M)
- Adjust BPAD (Time weighted average ($\mu$M))
- Establish in vitro free concentration of chemical ($\mu$M)
- Establish total chemical plasma concentration at steady state ($\mu$g/ml)
- External dose required to produce internal effect ($\mu$g/cm²)
- Percent in Product

Table:

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Formulation Percentage required to cause ‘toxic’ effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median product applied</td>
<td>39.58%</td>
</tr>
<tr>
<td>Worst case product applied</td>
<td>20.99%</td>
</tr>
</tbody>
</table>

Factors:

- Concentration, Skin penetration, Skin model
- Exposed area = Amt. of product applied * Exposed area
- Exposed area * Infusion rate * time
- PBPK model (fitted to in vivo data)
- Micronucleus, DNA repair centre assay, Transcriptomics
- Time concentration profile, AUC/time
- Chemical binding to serum (rapid equilibrium dialysis) TWA BPAD * percent free 2.6$\mu$M * 0.359
- Free TWA BPAD + Fraction unbound in vivo (literature or PBPK model) 0.28$\mu$g/ml ÷ 0.02
QUERCETIN RISK ASSESSMENT UNCERTAINTY

- Do the assays represent the biology?
- Have we captured or considered enough of the biology?
- What is the rationale for selecting the cell lines?
- What is the rationale for selecting doses and time points?
- What criteria is used to determine the BPAD (measurement/models)?
- Do we understand what is normal/what is adverse?
- Uncertainties
  - Cell line variability
  - Assay variability
  - Experiment variability
  - Distribution of BPAD values
- Uncertainties (BPAD application)
  - Biomarker function (in vivo)
  - Biomarker variation e.g. age, sex etc. *(in vivo)*
- Uncertainties (IVIVE)
  - difference in duration of exposure

Reverse Dosimetry (IVIVE)
Why do we have confidence in this approach?

**CONFIDENCE IN CURRENT SAFETY ASSESSMENT**

**Tox Endpoint**

“there’s an established quality of science, reporting standards & audit framework for studies that use in vivo models”

“animals are intact biological systems - a suitable model for the human system”

“not the ideal model of uncertainty but it is pragmatic”

NOAEL

NOAEL ÷ 10 - 1000

“there’s a broad level of scientific acceptance in the approach”

Safe Dose in Humans

“we’ve done it this way for decades and it seems to work”

“there's an established quality of science, reporting standards & audit framework for studies that use in vivo models”
HAVE YOU USED STANDARDS IN REPORTING?

Sources of Models:
- Developed internally or
- Academic collaboration
- Commercial software
- Literature models
Parameter Uncertainty
- Direct estimates from data
- Expert Knowledge Elicitation
- MC Simulation & Bayesian stats
- Quantitative uncertainty analysis

Model Uncertainty
- Empirical corroboration
- Assess model assumptions

Risk communication with probabilistic outcomes!
INCREASING CONFIDENCE IN PATHWAY BASED RISK ASSESSMENT

Evaluate uncertainties associated with integrating biokinetics and biodynamics:

- definition of BPAD/risk metric
- structure and code the risk assessment
- capture, document and rank uncertainties
- perform sensitivity/uncertainty analysis
- perform the final risk assessment with uncertainty explicitly represented and documented
SUMMARY/CONCLUSIONS

• Our aim is to understand how safety may be assured for complex toxicological endpoints using data derived from a toxicity pathways-based approach that is rooted in mechanistic understanding of the underlying biology.

• We performed pathway based risk assessment for the hypothetical case study by defining a BPAD, adjusting the BPAD to reflect *in vitro* biokinetics and finally we performed IVIVE.

• We need to investigate uncertainties around the approach to increase our confidence in using toxicity pathways for risk assessment:
  » BPAD estimation
  » Cell lines
  » Metabolism
  » Relating *in vitro* systems to *in vivo*
Unilever
Paul Carmichael
Matthew Dent
Sue Edwards
Paul Fowler
Stephen Glavin
Penny Jones
Moira Ledbetter
Sophie Malcomber
Beate Nicol
Andrew Scott
Sharon Scott
David Sheffield
Nikol Simecek
Alexandre Teixeira
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Susan Ross
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Patrick McMullen

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THANK YOU