CAN CANCER SAFETY ASSESSMENT BE CONDUCTED SOLELY ON THE BASIS OF IN VITRO STUDIES?

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INTERPRETATION OF NAS TT21C

High-throughput screening

Focused pathways approach
AOP FOR CANCER VS. SAFETY DECISION + 3RS

Hanahan & Weinberg, Cell 2000 + 2011
PROTOTYPE TOX PATHWAY: CHARACTERISE P53 DNA DAMAGE AT LOW DOSE

(1) activating DNA repair
(2) inhibiting cell cycle
(3) activating apoptosis

Bhattacharya et al., 2011 PLoS One 6(6)
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.
ORIGINAL HYPOTHESIS – MECHANISM FOR THRESHOLD BEHAVIOR

- **Homeostasis**
- **Adaptation**
  - p53 activation
  - Gene transcription
  - Cell cycle arrest
  - DNA repair
- **Adversity**
  - Micronuclei (mutation)
  - Apoptosis

The diagram shows the relationship between micronuclei (fold increase) and chemical dose, with different stages of response indicated by color-coded areas: green for homeostasis, purple for adaptation, and red for adversity.
TECHNOLOGIES

High-throughput flow cytometry (FACS)
- Protein expression in individual cells
- 96-well plates, up to 6 proteins simultaneously

High content imaging (HCl)
- Protein expression/localization
- 96, 384 well plates
- Up to 4 proteins simultaneously

Titan gene array
- Whole genome
- Simultaneous analysis of 96 samples
CHARACTERISING DOSE-DEPENDENT DNA DAMAGE PATHWAY

- HCl: Cellular response to DNA damage (toxicity pathway + case study chems)
  - Localization of Mn & DNA damage response proteins in single cells
    - phos-p53, total-p53, p21, MDM2, Chk2, p-ATM, H2AX

- High throughput flow cytometry (FACS)
- Alterations in gene expression following DNA damage
  - Time and dose-dependent changes
  - Full-genome arrays + ChIP-chip + ChIP-seq
Generic safety assessment:

» “Can chemical X be safely used at y% in product Z?”
» Focuses on providing reassurance that realistic chemical exposures will not significantly perturb cellular signalling pathways

Case study for QUE:

» Can QUE be safely used at 0.5% in lotion?
» Based on exposure assessment,
  • A person using 15 g body lotion/day => 75 mg QUE/day
» How do we use in vitro assays to determine whether this exposure is safe?
ESTIMATION OF QUERCETIN EXPOSURE IN CONSUMERS USING PBPK MODELS

In vitro skin penetration experiment and model

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Steady state conc. (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>400</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.016</td>
</tr>
</tbody>
</table>

PBPK model to estimate concentrations in plasma and other tissues
**PREDICTING ‘SAFE’ EXPOSURES**

*In vitro* adaptive/adverse threshold concentration (µM) – measuring & modelling FREE CONCENTRATIONS

- How do we make the link between *in vitro* hazard data and a risk to consumers?
IN VITRO MICRONUCLEUS (MN) ASSAY AS AN EARLY MEASURE OF ADVERSE OUTCOME

MN were evaluated for case study chemicals (18 doses) using high throughput flow cytometry and Cellomics™ in HT1080 cells (24 hrs). Changes in biomarkers were anchored to the MN assay.
CHARACTERISATION OF P53 PATHWAY OVER DOSE

High-throughput flow cytometry to measure pathway protein expression in cells

ETP induced a much stronger ATM response and was least successful at preventing permanent DNA damage i.e. MN

Transcriptomic to elucidate mechanism of action
- Micronuclei occur at lower doses than gene transcription

*BMDL

95% lower confidence interval for Benchmark Dose (Based on BM response of 1 SD)

*Associated enriched categories Development
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

Repair centre quantification, sensor kinase, PTM using phosphoproteomics

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

A POD or BPAD but accompanied by mechanistic rationale
Micronuclei occur at doses where DSBs are no longer efficiently repaired before cells divide.

**Adaptive**
- NOEL = 0.5 ng/mL

**Adverse**
- NOEL = 5 ng/mL

Resolved DNA repair centers (aka, successful repair)

Unresolved DNA repair centers

Micronuclei at 27 hr
- NOEL = 0.5
- NOEL = 5

p53BP1 - NCS (ng/ml)

Dose (ng/mL)

Response

0 10 20 30
5 10 15 20 25

p53BP1 Foci (foci/cell)

0 1 2 3 4 5 6 7 8
0 5 10 15 20 24 2.5 5 10 25

No MN

Yes MN

Unresolved DNA repair centers at (⇒ unrepaired DSBs)

Yes MN

No MN
ODE model for p53 pathway activation by ETP

The model (blue) recapitulates experimental data (red) and could be used to predict *in vitro* adverse outcome for certain chemicals.
SUMMARY, CHALLENGES & NEXT STEPS

Summary

Our aim has been 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.

Challenges and next steps

In vitro to in vivo extrapolation

Can we prove this theory? Is it conserved?

How can we make this flexible?
1. Adeleye et al. 2014, Toxicology
4. Sun et al. 2013, Toxicol. In Vitro
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FRAMEWORK

Chemical ingredient with ‘significant’ human exposure

In vitro HTS (pathway inference)  Chemical profiling (chemo-informatics)

Defined tox-pathway(s) of concern*

In vitro biokinetics & free concentration

In vitro adversity, point of departure (POD/BPAD) concentration determination

In vitro concentration response in appropriate assays

Chemical profiling (chemo-informatics)

Biokinetetic model (QIVIVE)

QSPR/in vitro physicochemical parameters

In vivo human safety estimate (mg/kg/day)

Computational systems biology models

1. Generic stress/toxicity pathways
2. Specific receptor-mediated pathways

In vivo HTS (pathway inference)

Biokinetetic model (QIVIVE)

QSPR/in vitro physicochemical parameters
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