PK and PD Tools for DNA-Damage Pathways: Modeling Dose Metrics and DNA-Repair Processes

Genetic Toxicology at the Crossroads
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Topics

Disclaimer about my limited qualifications at this or any genetic toxicology meeting

Risk assessment for carcinogens and mutagens – a personal perspective dating to 1971

Assessing risks/safety for mutagenic/carcinogenic compounds in a high throughput, TT21C (toxicity testing in the 21st century) world

PD/PK tools likely to play roles in safety testing with all compounds in the future
Creating the Risk Approach with Carcinogens

- In the late 1960’s the US initiated a “War on Cancer”

- Early 1970’s saw the establishment of USEPA

- In the Mid-1970’s Carcinogen Assessment Group establishes low dose and interspecies extrapolation for interpreting cancer bioassays

- Cancer incidence = $f$ (mutation potential) leads to explosion of in vitro mutagenicity tests

- Low dose linear modeling becomes set in concrete with the 2009 Report “Science and Decisions in Risk Assessment”
Serious Chinks in the Linear Low Dose Armor

- Studies with alkylating agents contaminants in anti-viral preparations – Lutz and Lutz modeling for non-linear response patterns

- Increasing sensitivity in looking at DNA and protein adducts. Under normal conditions there are many adducts of diverse types present on DNA bases

- Computational approaches to evaluating expected dose response to DNA-damaging or stress pathways provide strong basis for thresholds and perfect adaptation.
Now we’re Standing at the Crossroads

From Qualitative Hazard Evaluation to Quantitative Risk Assessment

In general, genetic toxicology has focused on technology of the assays rather than evaluating risks posed to populations by exposure.

New tools are coming along to do high throughput testing of the ability of compounds to activate DNA repair networks.

We need to have an appropriate suite of PD and PK tools available to interpret these data streams for risk/safety not simply hazard.
DNA-Damage Pathways

- Treatment of Cells
  - Absorption
  - Biologic Interaction
  - Perturbation

- Biologic Inputs
  - Higher Concentration

- Increased Strand Breaks

- Normal Biologic Function

- Increased Repair

- Cell Injury
  - Mutation
  - Apoptosis
  - Cell cycle Arrest

- Higher yet

Low Concentration
Predicting safe exposures from *in vitro* assays – gene tox or any others

- Assessing adversity *in vitro*
- Computational Systems Biology Pathway (CSBP) Modeling

- *In vitro* assays for specific toxicity pathways
- Point of Departure (concentration)
- Acceptable concentration *in vitro* (ug/l)

*In vitro-in vivo* dosimetry PK Modeling

*In vivo* human exposure ‘standard’ mg/kg/day
Pathway dose response goes from regions without changes from background, through adaptive and on to adverse outcomes.

What assays are necessary to measure adaptive and adverse responses?
Defining key assay readouts for the p53 pathway

- ATM
- DNA-PK
- ATR
- p38
- WIP1
- MDM2

- DSB
- SSB
- Other damage

- Apoptosis
- Cell Cycle
- DNA repair

Escape from control: MUTAGENICITY
Whole genome transcriptomics – Chemical comparison

Pathway profiling across chemicals

Dose-response

Methyl Methanesulfonate

EC$_{50}$ = 59.7 µM

Concentration (µM)

Total p53 % Responder

Total p53

0 20 40 60 80

0.0001 0.001 0.01 0.1 1 10 100

Concentration (µM)
## Comparison of dose-response across endpoints

<table>
<thead>
<tr>
<th>BMDL</th>
<th>ETP</th>
<th>QUE</th>
<th>MMS</th>
</tr>
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<tr>
<td>pH2AX</td>
<td>0.05</td>
<td>9</td>
<td>80</td>
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<tr>
<td>p-p53 (s15)</td>
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<tr>
<td>Tp53</td>
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<td>7</td>
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<tr>
<td>Cell Cycle</td>
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<td>51</td>
<td>61</td>
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<tr>
<td>Apoptosis</td>
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<td>p-p53 (s46)</td>
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<tr>
<td>HCl-Micronucleus</td>
<td>0.03</td>
<td>1</td>
<td>19</td>
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<tr>
<td>Gene Transcription*</td>
<td>0.1</td>
<td>8</td>
<td>25</td>
</tr>
</tbody>
</table>

*Associated enriched categories are Immune Response, Development and Cytoskeleton Remodeling for ETP, QUE, MMS
Assays Looking at DNA Repair

- RIFs formed in higher yield at lower doses
- Assuming direct proportionality between DSB and radiation dose:
  1.0 DSB/RIF at 0.4 Gy
  2.3 DSB/RIF at 2.0 Gy

Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells
Neumaier et al., PNAS, 2012.
DNA repair centers

24h

Con

1 uM ETP

NCS 400 ng/mL

p-H2AX
p53 BP1
Overlay
Quantitation of repair centers

neocarzinostatin

etoposide
Interpreting DNA damage dose-response data

- NOEL = 0.5 ng/mL
- NOEL = 5 ng/mL

**Induced DNA repair centers (induced DSBs)**

**Unresolved DNA repair centers at 24 hr (\(\approx\) unrepaired DSBs)**

![Graph showing dose-response data with NOEL values and labels for induced and unresolved DNA repair centers.](image-url)
Interpreting DNA damage dose-response data

Resolved DNA repair centers
(aka, successful repair)

Induced DSBs

Unrepaired DSBs

NOEL = 0.5 ng/mL

NOEL = 5 ng/mL
Interpreting DNA damage dose-response data

Adaptive response, i.e., “safe region”

Induced DSBs
Unrepaired DSBs

NOEL = 0.5
NOEL = 5
ETP shows almost no repair, indicating that MN and foci induction should occur at similar doses. This behavior is consistent with the mode of action where ETP blocks topoisomerase and repair processes.
Low Dose Linear or Thresholds

Contrary to expectations from linear low-dose risk assessment defaults, experiments now provide strong evidence for non-linear low-dose behaviors, even ‘thresholds’ for mutational responses

Are “thresholds” consistent with expectations based on control theory and the structure of stress pathway negative feedback networks?
The functional repertoire of a network is determined by its topology. Ma et al. (2009) analyze enzyme networks with three nodes and take a reverse-engineering approach to ask how many core network topologies can establish perfect adaptation, the ability to reset after perturbation. Surprisingly, the answer is just two.
Post-translational and transcriptional routes for DNA-repair processes

UV → ATR → Chk1 → p53 → Post-translational feedback

DSBs → ATM → Chk2 → p53 → Transcriptional Control

p53

ATM

H2AX

BRCA1

Post-translational feedback

Transcriptional Control
How could we achieve a threshold for mutation following low level damage?

Homeostasis likely requires perfect adaptation of both rapidly acting pathways (post-translational modification) and slower acting pathways (transcriptional).

Set POD at concentration where cell cannot prevent adverse effects.

\[ \text{micronuclei} = f(Y) \]
Lower doses - the model produces 'perfect' control through **zero-order dephosphorylation** of ATM-Pi and activation of p53 by ATM-Pi – an example of post-translational homeostasis.

Model schematic:

- **DS-DNA** → **DSB** → **(γ−H2AX)** → **Repair Centers** → **DS-DNA**

- **ATM** ↔ **ATM-Pi**
- **p-53** ↔ **p-53-Pi**

DNA-repair genes

Model schematic image:

![Model schematic diagram](image-url)
How might this information be used for risk/safety assessments with DNA-damaging compounds?
“Predictive” Toxicology:
From Qualitative Hazard Evaluation to Quantitative Risk Assessment

- Possibility of a response
  - many measures might work
- Expected incidence of specific response
  - reproduce all key mode of action
  - do we ever know them all
- No reasonable likelihood of response
  - focus on key events that are essential
  - estimate doses without effects
The PK side of IVIVE

- Concentration in cells
- Concentration of metabolites
- Rates of metabolism

Measure of Adversity (in vitro concentration)

Point of Departure (in vitro concentration)

reverse dosimetry

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

toxicity pathway assays

cell-based pathway modeling
Reverse dosimetry relates the Concentration in an *in vitro* Assay to an Equivalent *in vivo* Human Exposure

*In Vitro Toxicity Assays*
Using a simplified approach for starters

Hepatocellular Clearance

Plasma Protein Binding

Estimated Renal Clearance

Prediction of in vivo clearance

Concentration from in vitro assay

Reverse Dosimetry

Equivalent Oral Exposure
An Implementation with HTS showing utility of reverse dosimetry

By themselves, in vitro assay effect concentrations are not quantitatively useful for risk assessment

(Rotroff et al. 2010; Wetmore et al., 2012)
Need tools to create generic Physiologically Based Pharmacokinetic Models for more diverse exposures
Some questions as we stand with the Devil at the Gene Tox Crossroads?

- How many damage pathways do we need to assess?

- What assays are best for looking at repair – e.g., real time foci analyses for single cells?

- How do we engage with an entrenched risk assessment bureaucracy to change current methods?

- Can we find case studies that allow comparison of in vitro assay based DNA-damage compound safety assessments with conventional approaches?

- Is the discipline of toxicology testing and risk assessment willing to do the hard work for change?
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Cells are not just passive, when a stressor comes along. They detect that there is trouble and adapt to what’s goin’ on. Tell me how do they do it? I don’t see how cells do it. Tell me how do they do it – react that way?

Well, ho-omeostasis is a mystery to me. How do cells know how they should be and avoid toxicity? Tell me how do they do it? I don’t see how cells do it. Tell me how do they do it – react that way?

With double negative feedback, feed forward and cascades, Cells can act like switches when a change needs to be made. Tell me how do they do it? I don’t see how cells do it. Tell me how do they do it – react that way?

Hormesis ain’t no fiction, it’s just stimulus-response. With feed forward cells can act fast and beat trouble to the punch. Tell me how do they do it? I don’t see how cells do it. Tell me how do they do it – react that way?

When their DNA is damaged, cells stop cycling till it’s fixed. But if things get out of hand then suicide is what they picks. Tell me how does they do it? I don’t see how cells do it. Tell me how do they do it – react that way?

**Systems Toxicology is the way we need to go**
To stop relying on animal tests and learn what we need to know
Tell me why don’t we do it? I don’t see why we can’t do it.
Tell me why don’t we do it – find a better way?

Tell me how do they do it? *(How do they do it?)*
Tell me how do they do it? *(How do they do it?)*
Tell me how do they do it? *(How do they do it?)*
I don’t see how cells do it. *(How do they do it?)*
Tell me how do they do it – react that way?