Hypothesis-based testing for developmental toxicity

George Daston
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Attention: Dr. Fred H. Snyder

Gentlemen:

During the past several years following the thalidomide episode, we have been recommending a study designed to determine the potential of drugs for producing adverse effects on the reproductive process. The guidelines for this study reflected a modification of a test used for many years by the food industry to provide evidence of safety of food additives. The introduction of the two-litter test appeared to offer a reasonable approach to the over-all problem of assessing the safety of drugs on reproduction. It was anticipated that the two-litter test would prove an adequate screening procedure for the elucidation of adverse effects of a new drug on the reproductive process and that such effects could be subjected to a critical evaluation.
modifications be necessary, they can be instituted earlier. Of paramount importance, of course, is that studies designed along the lines of our new recommendations should yield more meaningful data upon which to base an evaluation of safety.

It must be realized that even these improved guidelines reflect merely the "state of the art" at the present time, and undoubtedly further modifications will be needed in the future as additional knowledge in this area is developed. We hope these suggestions will prove helpful.

Sincerely yours,

[Signature]

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Enclosure
Overview

- What is hypothesis-based testing?
- Selecting the best models and approaches based on what is known about mode of action
- Predictive toxicity workflow based on computational and biotechnology tools
Hypothesis-driven testing

• Generate hypotheses about how an agent will affect development
  – Chemical similarities to chemicals already tested
    • 2D structure, phys chem properties, reactivity, interaction with specific proteins
  – Functional similarities to chemicals already tested
    • Same target, similar results in gene expression, HTS

• Select models and protocols based on hypothesis to be tested
Predicting Toxicity

- Adverse responses at the organismal level must be underpinned by responses at the molecular and cellular level.
- It is becoming increasingly possible to measure potential molecular and cellular effects globally.

From AOP-KB
Areas of certainty and uncertainty

Many available methods
- QSAR
- HTS (ToxCast)
- toxicogenomics

High uncertainty about
- Which key events
- Non-linear relationships
  - Quantitative thresholds
  - Interacting pathways

Lots of historical data
Risk Assessment by Analogy

Animal Toxicity Data

- Chemical similarity
- Common metabolism
- Common MOA

Dose-response

BMD

- PK adjust

- UFs (Confidence adjust)
  - TK
  - TD variability

Acceptable Level

Analog
Predictive Toxicology workflow

- Cheminformatics
- High quality analogs
- More data needed
- Mechanistic models
- Systems biology models
- PK Models
- Decision
- Exposure
Sixty Years of Toxicology Data

• Databases that have toxicology (or at least relevant) data on 800,000+ chemicals
• DART data: 23,000+ chemicals
• Database searchable by chemical structure
• Analysis of the toxicology data is still expert-driven
Output – Substructure Searching
Analogs as hypothesis generation

• Analogs have similar toxicity because one of the following is true:
  – They share a common metabolite, or one is metabolized to the other (e.g., the acetate ester of EGME has the same toxicity as EGME because it is hydrolyzed to EGME)
  – They have the same biological activity
    • Can be tested if MOA is known
    • Can be tested in a more global system if MOA is not known
Predictive Toxicology workflow

- Cheminformatics
- Mechanistic models
- PK Models
- Systems biology models
- Exposure
- Decision
How many MOAs?

• Unknown, but finite

• An expansion of the druggable genome
  – Macromolecular targets for small molecules that change the function of the macromolecule or cause its normal function to be excessive or inadequate
    • Less than 10% of genome
  – Add chemical reactivity, non-protein targets

• Can be estimated by retrospective literature analysis
<table>
<thead>
<tr>
<th>Generic category</th>
<th>Main category</th>
<th>Sub-category 1</th>
<th>Sub-category 2</th>
<th>Prototype chemical</th>
<th>Prototype structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor and enzyme-mediated toxicity</td>
<td>Nuclear hormone receptor ligands</td>
<td>Estradiol-like</td>
<td>17-beta-estradiol</td>
<td>Phytoestrogens and flavones</td>
<td>genistein</td>
</tr>
</tbody>
</table>
Testing at an MOA level

• Requirement is for broad coverage
  – HTS batteries with broad coverage
  – Global gene expression analysis
    • Need to have an appropriate number of cell types for broad coverage
Inferring common MOA from gene expression
Daidzein : (MCF-7)

Top 20:
Steroidal estrogens: 9
Androgen/progestagen: 4
Phytoestrogen/ polyphenol: 5
Reserpine (MCF-7)

- Inhibits vesicular monoamine (dopamine, serotonin, norepinephrine) transporter
- 10 of top 20 have an effect on the transporter or inhibit monoamine receptors
- An additional two are monoamine reuptake inhibitors
Conclusions

• Hypothesis-driven toxicity testing requires a lot of data to ensure that the range of possible MOAs has been covered, but the data are increasingly available
  – 60 years of testing at the organismal level
  – Increasing understanding of the universe of possible modes of toxicity (the “intoxicable” genome)
  – Techniques that provide global coverage
  – Computational power to find appropriate data (and to create and test systems-level models)
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