Threshold-Based Risk Assessment is the Same for Cancer and Non-cancer Endpoints for Non-DNA Reactive Carcinogens

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Conflict of Interest Statement

- Member of FEMA Expert Panel
- US EPA Science Advisory Board
- Consult for several pharmaceutical and chemical companies
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Presentation Outline

• Carcinogenesis
• Mode of action/human relevance analysis
• Screening for cancer risk
• Non-genotoxic carcinogenesis
• Inorganic arsenic
• Conclusion
What We Know

• Genetic alterations required for cancer formation
• More than one genetic alteration required
• DNA replication fidelity is not 100%
• Cancer arises from stem cell population
• Cancers are clonal
• Carcinogenesis is stochastic process
Means of Increasing Risk of Cancer

• Increase rate of DNA damage per cell division
• Increase number of cell divisions
Modes of Action of Human Carcinogens

- DNA Reactive
- Immunosuppressive
- Estrogenic
- Cytotoxicity and regeneration
### Increased Cell Proliferation and Carcinogenesis

#### DIRECT MITOGENICITY

<table>
<thead>
<tr>
<th>Event</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR, PXR activation</td>
<td>liver</td>
</tr>
<tr>
<td>PPARα activation</td>
<td>liver</td>
</tr>
<tr>
<td>AhR activation</td>
<td>liver</td>
</tr>
<tr>
<td>T4, T3 metabolism → TSH stimulation</td>
<td>thyroid</td>
</tr>
<tr>
<td>Cholecystokinin activation</td>
<td>rat pancreas acinar cell</td>
</tr>
<tr>
<td>Proton pump inhibition → Gastrin activation</td>
<td>gastric carcinoid</td>
</tr>
<tr>
<td>Prolactin activation</td>
<td>rat mammary</td>
</tr>
<tr>
<td>Estrogen increase</td>
<td>mammary</td>
</tr>
<tr>
<td>LH stimulation</td>
<td>rat testicular Leydig cell</td>
</tr>
<tr>
<td>Club cell mitogenesis</td>
<td>mouse lung</td>
</tr>
</tbody>
</table>

#### CYTOTOXICITY & REGENERATION

<table>
<thead>
<tr>
<th>Event</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary solids</td>
<td>bladder</td>
</tr>
<tr>
<td>Hepatocellular necrosis</td>
<td>liver</td>
</tr>
<tr>
<td>Kidney necrosis</td>
<td>kidney</td>
</tr>
<tr>
<td>α₂u-globulin → toxicity</td>
<td>rat kidney</td>
</tr>
<tr>
<td>Chronic progressive nephropathy</td>
<td>rat kidney</td>
</tr>
<tr>
<td>Forestomach irritation</td>
<td>forestomach</td>
</tr>
<tr>
<td>Cytotoxic urinary metabolites</td>
<td>bladder</td>
</tr>
<tr>
<td>Iron accumulation</td>
<td>liver</td>
</tr>
<tr>
<td>Tubular apoptosis</td>
<td>kidney</td>
</tr>
<tr>
<td>Bronchoalveolar necrosis</td>
<td>lung</td>
</tr>
</tbody>
</table>
Basic Assumptions of Animal Bioassays for Human Risk Assessment:

1. Carcinogenic effects at high doses will also occur at low doses (dose extrapolation).

2. Chemicals that cause cancer in rodents will cause cancer in humans (species extrapolation).

1. Is the weight of evidence sufficient to establish the MOA in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between experimental animals and humans?
3. Can human relevance of MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?
4. Statement of confidence; analysis; and implications
Two-Year Rodent Bioassay

- Cost: time, money, animals
- Dose response: limited
- Mode of action: not determined
- Human relevance: can’t evaluate
Detailed 4 & 13-Week Bioassays

- Organ Weights
- Histologic Evidence of Toxicity and/or Proliferation
- Blood and Urine Chemistries
- DNA Labeling Indices
- Specialized Studies
  - Colon Roll – Aberrant Crypt Foci
  - Immunohistochemistry
  - Omics?
Rodent Tumors Not Relevant to Humans

- Rodent organs without human counterpart
  - Zymbal’s gland
  - Harderian gland
  - Forestomach

- Rodent tumors without human analog
  - Spenic mononuclear cell leukemia
  - Mouse submucosal mesenchymal lesion of bladder (seminal vesicles, uterus)

- Endocrine organs
  - Thyroid
  - Adrenal cortex
  - Adrenal medulla
  - Pituitary – anterior
  - Pituitary – posterior
  - Parathyroid
  - GI endocrine cells
  - Pancreatic islets

- Reproductive endocrine tumors
  - Ovary – granulosa cell
  - Testis – Leydig cell (? Mesothelioma)
  - Endometrium
  - Prostate
Screening for Carcinogenesis

Chemical

- DNA Reactive
  - Yes: Short term in vivo assay at MTD to identify possible target tissues. Possible human carcinogen; requires risk assessment
  - No: 13-week bioassay screen to evaluate cytotoxicity and/or ↑ cell proliferation

- Immunosuppressive
- Estrogenic activity

- Yes: Possible human carcinogen; requires risk assessment
- No: Unlikely human carcinogen for intended use and expected exposure

Specific evaluation to determine MOA and dose response in tissues positive in screen

- MOA and dose relevant to humans: Possible human carcinogen; requires risk assessment
- No: Unlikely human carcinogen for intended use and expected exposure
Follow-Up Detailed Studies

- Dose Response (Expand number of Doses)
- Metabolism – Non-linearities?
- Toxicokinetics
- Mode of Action
It’s Time to Stop Doing Two-Year Rodent Bioassays
Non-genotoxic Carcinogenesis

Chemical Exposure

Non-cancer Toxicity

Increased Cell Proliferation

Tumors
Non-genotoxic Carcinogens

- Precursor toxicity lesion necessary
  - Mitogenicity
  - Cytotoxicity and regeneration
- Dose response involves a threshold
  - Same as non-cancer toxicities
- Protecting against precursor non-cancer toxicity will protect against cancer
Inorganic Arsenic in Drinking Water

• High exposure levels (>150 ppb) related to cancer
  − Skin
  − Urinary bladder
  − Lung
  − Kidney (kidney pelvis, same as bladder)
  − Liver?
Classic Pathway for Arsenic Metabolism

Putative Pathway for Arsenic Metabolism

Trivalent Arsenicals Interacting with Sulfhydryl Groups

Pentavalent  Trivalent  Protein Interaction

Arsenate (iAs^V) → Arsenite (iAs^III) → R-SH → R-S-As
MMA^V → MMA^III → Me
DMA^V → DMA^III → Me
TMA^IVO →

Tsuji, et al., in press
Urothelial Cytotoxicity and Proliferation Induced By Inorganic Arsenic

Cohen et al., 2013
Mode of Action for Inorganic Arsenic Carcinogenesis

- Key Events
  - Ingestion of significant amounts of arsenic
  - Generation of trivalent forms (iAsIII, MMAIII, DMAIII)
  - Reaction with critical cellular thiols (glutathione, proteins)
  - Cytotoxicity and cell death
  - Regenerative proliferation
  - Tumors
Relevance To Humans

URINARY BLADDER

- Clinical Manifestations and Arsenic Methylation After a Rare Subacute Arsenic Poisoning Accident
  - High exposure
  - DMAV & TMAO in urine
  - Hematuria in 1/3 of exposed group

HUMAN CHRONIC BRONCHITIS

SKIN: ACTINIC KERATOSIS

Cohen et al., 2013
Implications for Risk Assessment

Trivalent arsenicals

Threshold

Non-cancer Biological Effects

Skin arseniasis

Bronchial toxicity

Urothelial toxicity

Cancer

Other non-cancer toxicities

Sulfhydryl groups

Cohen et al., 2013
Mode of Action for Inorganic Arsenic: Working Hypothesis

- Ingestion of Arsenic
- Conversion to Trivalent Arsenicals (iAs$_{III}$, MMA$_{III}$, DMA$_{III}$)
- Interaction with Critical Cellular Constituents with Sulphydryl Groups
  - Threshold
  - Cellular Effect
    - Certain Epithelia
      - Cell Death
      - Regenerative Proliferation
      - Precancerous Lesions
      - Carcinoma
    - Other Tissues
      - Adverse Cellular Events
        - Non-Cancer and Non-Precancerous Effects

* Due to constant turnover of cellular constituents with sulphydryl groups such as proteins

Cohen et al., 2013
Odds Ratios for Lung and Bladder Cancer at Low-level Arsenic Exposures

Tsuji et al., in press
Among participants with confirmed skin lesions for whom we had complete water histories, the lowest known peak arsenic concentration ingested by a case was 115 µg/liter.

Haque et al., Epidemiology, 14:174, 2003
Inorganic Arsenic in Drinking Water

• Mode of action involves cytotoxicity and regeneration
• Mode of action involves a threshold dose response
• Protecting against cytotoxicity precursor lesions will protect against cancer
• Threshold for humans is between 50 and 150 ppb
Non-genotoxic Carcinogens

- Mode of action involves increased cell proliferation
- Precursor proliferative lesion leads to cancer over time (probabilistic); is a necessary step
- If precursor lesion isn’t produced, no cancer risk
- Protecting against non-cancer endpoint protects against cancer
- Screening for Precancer Events Protects Against Cancer
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