Qualitative and Quantitative Approaches in the Threshold of Genotoxic Carcinogens

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Present Concept of Chemical Carcinogenicity

Carcinogenic response

Non-genotoxic carcinogen

NOEL

Genotoxic carcinogen

No threshold (LNT)
Low-dose Carcinogenicity Study of 2-Acetylaminofluorene (Megamouse Experiment)

- Animal: 24,192 female BALB/c mouse, 3-4 weeks of age
- Doses of 2-acetylaminofluorene (2-AAF) in diet: 0, 30, 35, 45, 60, 75, 100, 150 ppm
- Time of sacrifice: 9 ~ 33 months

Do not contradict “No Threshold” theory

Dose model for bladder neoplasm

Dose model for liver neoplasm

(Famer J.H., et al., J Environ Pathol Toxicol, 1979, 3: 55-68)
Reconsideration of Linear Non-threshold Theory

Low-dose carcinogenicity curve of genotoxic carcinogens: Extrapolation from high to low doses

It has been argued that non-threshold theory is challenged based on the view that organism possess biological responses that can ameliorate genotoxic activities.
Extrapolation of Genotoxic Carcinogenicity Study Results to Human

- Qualitative analysis only to classify into genotoxicity or non-genotoxicity is inadequate for carcinogenic risk assessment
- Qualitative and quantitative assessments are desirable in analysis for carcinogenicity, particularly at low doses
- Weight of evidence: *in vivo* data are more valuable than *in vitro* results in the quantitative analysis
- Point of departure (PoD) can be used for quantitative analysis of genotoxicity and carcinogenicity dose-response data
- PoD in markers of *in vivo* carcinogenic mechanism may contribute to resolution of putative non-threshold theory of genotoxic carcinogens
Chemical Carcinogenesis Mechanisms

Carcinogen

- Metabolic activation: ultimate carcinogen
- DNA adduct formation
- Oxidative stress
- DNA repair error
- DNA repair
- Inactivation

Non-DNA

- Cancer-irrelative mutations
- Apoptosis

Mutation

- A→C mutation

Initiation

- GST-P positive foci
- GST-P positive cell (possibly mutated cell)

Promotion

- Cancer
- Premalignancy: cell proliferation
- Apoptosis

Cancer:

- Malignancy
- Irreversible change
- Preneoplasia: cell proliferation
- Apoptosis

Inactivation

- Non-DNA
MelQx

One of heterocyclic amines
- Exists in well-cooked fish and meat
- Genotoxicity: Ames test, positive
- Chromosome aberration test, positive
- Structural aberration: positive
- Hepatocarcinogen
- Human exposure level: 0.2-2.6 µg/day
- IARC category: 2B
  (probably carcinogenic to humans)

Hepatocarcinogenicity in rats

Liver tumor incidence (%)

<table>
<thead>
<tr>
<th>MelQx dose (ppm in diet)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P<0.01

(Wakabayashi K., et al., Carcinogenesis, 1996, 17: 1029-1034)
Rat Hepatocarcinogenicity of MelQx at Low Doses

Animals: 1,180 male F344 rats, 21-day-old

MelQx-DNA adduct

* p<0.05

4 wks

MeIQx dose (ppm in diet)

Adduct /10^7 ntd

8-OHdG /10^5 dG

* p<0.01

8-OHdG

NOEL, ND
BMDL10, 2e-05
BMDL1SD, 4.06

0.01 0.1 1 10 100

0.01 0.1 1 10 100

0.001 0.01 0.1 1 10 100

NOEL, 0.1
BMDL, 0.07

Number (/cm²)

GST-P positive foci

* p<0.01

32 wks

16 wks

0 0.001 0.01 0.1 1 10 100

0 0.001 0.01 0.1 1 10 100

(Fukushima S., et al., JJCR, 2002, 93: 1076-1082)
Incidence of LacI Gene Mutations and Development of GST-P Positive Foci in the Liver of Big Blue Rats Treated with MeIQx for 16 Weeks

**Incidence (No./10^6)**

- **LacI gene mutations**
  - NOEL, 1
  - BMDL10, 0.08
  - BMDL, 6.98

- *p<0.01

**Number (No./cm^2)**

- **GST-P positive foci**
  - NOEL, 10
  - BMDL, 6.72

* *p<0.01

**LacI gene:** 30~40 copies on chromosome 4 in the F344 rat

Initiation Activity of MeIQx at Low Doses in the Rat Liver

Animals: 850 male F344 rats, 21-day-old

Phenobarbital, 500 ppm in diet

MeIQx; 0, 0.001, 0.01, 0.1, 1, 10, 100 ppm in diet

Number (No./cm²)

GST-P positive foci

* p<0.01

NOEL, 1
BMDL, 10.97

Rat Heatocarcinogenicity of MeIQx in Long-term Carcinogenicity Test

Liver tumors (54 wks)

<table>
<thead>
<tr>
<th>MeIQx (ppm)</th>
<th>No. of rats</th>
<th>Incidence (%)</th>
<th>Hepatocellular adenoma</th>
<th>Hepatocellular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>5 (17) *</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>29</td>
<td>13 (45) *</td>
<td>13 (45) *</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>19</td>
<td>1 (6)</td>
<td>15 (94) *</td>
<td></td>
</tr>
</tbody>
</table>

Liver tumors (104 wks)

<table>
<thead>
<tr>
<th>MeIQx (ppm)</th>
<th>No. of rats</th>
<th>Incidence (%)</th>
<th>Hepatocellular adenoma</th>
<th>Hepatocellular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>51</td>
<td>14 (27) *</td>
<td>6 (12) *</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.01 v.s. 0 ppm

GST-P positive foci (104 wks)

Adenoma: NOEL, 1
BMDL, 22.54

Carcinoma: NOEL, 1
BMDL, 44.54

(Kushida H., et al., Cancer letters, 1994, 83: 31-35)

### Markers of MelQx Rat Hepatocarcinogenesis and the Comparison with Point of Departure (PoD)

<table>
<thead>
<tr>
<th></th>
<th>DNA adduct</th>
<th>Mutation</th>
<th>GST-P⁺ Foci</th>
<th>Adenoma</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NOEL</strong></td>
<td>ND</td>
<td>1</td>
<td>10</td>
<td>&lt; 100</td>
<td>100</td>
</tr>
<tr>
<td><strong>BMDL10</strong></td>
<td>2e-05</td>
<td>0.08</td>
<td>0.14</td>
<td>11.4 (tumors)</td>
<td></td>
</tr>
<tr>
<td><strong>BMDL</strong></td>
<td>4.06</td>
<td>6.98</td>
<td>15.12</td>
<td>60.25</td>
<td>72.69</td>
</tr>
</tbody>
</table>

ND, not detected

BMDL ranking: DNA adduct < Mutation < Preneoplasia < Tumor
# Preneoplastic Lesions or Tumors in MelQx Rat Hepatocarcinogenesis and the Comparison with PoD

<table>
<thead>
<tr>
<th>GST-P⁺ Foci</th>
<th>Adenoma</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>104 wks</td>
<td>54 wks</td>
<td>104 wks</td>
</tr>
<tr>
<td>NOEL</td>
<td>&lt; 100</td>
<td>1</td>
</tr>
<tr>
<td>BMDL</td>
<td>44.52</td>
<td>60.25</td>
</tr>
</tbody>
</table>

**BMDL ranking:** Adenoma < Carcinoma (54 weeks, 104 wks)  
Adenoma < Preneoplasia & Carcinoma (104 wks)
Risk of Liver Cancer: Reaction Curves for the Carcinogenicity Markers Dependent on the Dose of MeIQx

- DNA adduct < Mutation < Preneoplasia < Tumor
- Existence of a carcinogenic threshold
MelIQx DNA Adduct Levels and Number of GST-P Positive Foci in the Liver of Rats under Damaged Liver Condition

MelIQx DNA adduct level (x10^-7)

- TAA+MelIQx: NOEL, ND
  - BMDL, 1.46
- MelIQx: NOEL, ND
  - BMDL, 3.02

MelIQx-DNA adduct level vs MelIQx dose (ppm)

- ** P < 0.01 vs 0.1 ppm

GST-P positive foci

- TAA+MelIQx: NOEL, 1
  - BMDL, 9.68
- MelIQx: NOEL, 16
  - BMDL, 32.45

No. of GST-P positive foci vs MelIQx dose (ppm)

- ** P < 0.1 vs MelIQx
- a, bP < 0.01 vs 0 ppm

Male 280 F344 rats, 21-day-old

PoDs of DNA Adduct and GST-P Positive Foci in MeIQx Rat Hepatocarcinogenesis under Damaged Liver Condition

<table>
<thead>
<tr>
<th>DNA adduct</th>
<th>GST-P+ Foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAA-MeIQx</td>
<td>1.46</td>
</tr>
<tr>
<td>MeIQx</td>
<td>3.02</td>
</tr>
<tr>
<td></td>
<td>9.68</td>
</tr>
<tr>
<td></td>
<td>32.45</td>
</tr>
</tbody>
</table>

BMDL values: TAA→MeIQx < MeIQx
BMDL ranking: DNA adduct < Preneoplasia
Food-derived heterocyclic amine

Mutagenicity: positive

Genotoxicity (Chromosome aberration test):

Structural aberration: positive

Carcinogenicity in male rats: liver, colon, etc.

(300 ppm in diet, 2-year carcinogenicity study)

IARC category: 2A

2- amino-3-methylimidazo[4,5-f]quinoline
Induction of DNA Adduct and GST-P Positive Foci in the Livers of Rats Administered IQ for 16 weeks

<table>
<thead>
<tr>
<th>IQ (ppm)</th>
<th>No. of rats</th>
<th>GST-P positive foci (No./cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>240</td>
<td>0.15 ± 0.31</td>
</tr>
<tr>
<td>0.001</td>
<td>240</td>
<td>0.16 ± 0.31</td>
</tr>
<tr>
<td>0.01</td>
<td>240</td>
<td>0.26 ± 1.30</td>
</tr>
<tr>
<td>0.1</td>
<td>240</td>
<td>0.15 ± 0.35</td>
</tr>
<tr>
<td>1</td>
<td>240</td>
<td>0.14 ± 0.33</td>
</tr>
<tr>
<td>10</td>
<td>240</td>
<td>0.74 ± 0.88 *</td>
</tr>
<tr>
<td>100</td>
<td>120</td>
<td>88.03 ± 50.41 *</td>
</tr>
</tbody>
</table>

* p<0.01 v.s. 0 ppm

NOEL, 1
BMDL, 61.96

IQ-DNA adduct

0, 0.001 ppm: under detection limit (/5x10^10 ntd)

NOEL, ND
BMDL, 0.09

(Wei M., et al., Cancer Sci., 2010, 102: 88-94)
## Relationship between Markers in IQ Carcinogenesis of Rat Livers and PoD

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</thead>
<tbody>
<tr>
<td>NOEL</td>
<td>ND</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>BMDL</td>
<td>0.09</td>
<td>1.22</td>
<td>61.96</td>
</tr>
</tbody>
</table>

BMDL ranking: DNA adduct < Mutation < GST-P⁺ Foci
Reaction Curves for the Carcinogenicity Markers Dependent on the Dose of IQ

Control level

Response

IQ-DNA adduct

IQ doses

Gene mutation

GST-P positive foci

Liver cancer

DNA adduct < Mutation < Preneoplasia < Tumor

Existence of a carcinogenic threshold
Conclusions

✓ In qualitative analysis, Mode of Action in genotoxic carcinogens is an important tool for the analysis of low dose carcinogenicity.

✓ In quantitative analysis, PoD is a useful tool for the determination of exposure level in each marker of carcinogenesis. BMD may be an appropriate method.

✓ In MeIQx or IQ carcinogenicity, values of PoD were different and increased in order of DNA adduct, mutation, GST-P positive foci and tumor.

✓ These data will contribute to understand whether genotoxic carcinogenic threshold exists or not.
Collaborators

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