Existence of Carcinogenic Threshold: Evidence from Mechanism-Based Carcinogenicity Studies

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Environmental carcinogens

- Genotoxic or non-genotoxic
- Natural or synthetic
- Cooking process, contamination, or synthesis in the body
- Avoidable or unavoidable
- Human intake, 1.5 g/day (B. Ames)

Genotoxic

Non-genotoxic

Chemical reaction:

\[
2\text{HNO}_2 \leftrightarrow \text{N}_2\text{O}_3 + \text{H}_2\text{O}
\]

\[
\text{RCH}_2 + \text{R'}\text{CH}_2 \rightarrow \text{NH} + \text{N}_2\text{O}_3
\]

\[
\text{RCH}_2 + \text{R'}\text{CH}_2 \rightarrow \text{N} \rightarrow \text{NO} + \text{HNO}_2
\]

N-nitrosamine
Present concept of chemical carcinogenicity

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

- It is generally considered that genotoxic carcinogens have no threshold in carcinogenic potential. This hypothesis has led to acceptance of linear curve that approach zero at low doses for risk assessment. There are, however, limited date available for these hypothesis.
- It has been argued that non-threshold theory is challenged based on the view that organism possess biological responses that can be ameliorate genotoxic activities.
- Therefore, it is important to resolve this question from the view point of cancer risk assessment and management.
Merit of a medium-term bioassay for carcinogens

Normal tissue → Preneolastic lesion → Benign tumor → Cancer

Liver medium-term bioassay

Number-Area / unit of glutathione S-transferase placental form (GST-P) positive foci

Carcinogenicity test

Incidence of tumors
Chemical carcinogenesis mechanism

Carcinogen

Inactivation

Non-DNA

DNA repair

Metabolic activation: ultimate carcinogen

DNA adduct formation

Oxidative stress

DNA repair error

Mutation: irreversible change

Cancer-irrelative mutations

Apoptosis

Preneoplasia: cell proliferation

Cancer: malignancy

Initiation

Promotion

GST-P positive foci

http://www.intelihealth.com
MeIQx

One of heterocyclic amines
 Exists in well-cooked fish and meat
 Mutagenicity: positive
 Hepatocarcinogen
 Human exposure level: 0.2-2.6 µg/day

MelIQx: 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline

(Wakabayashi et al, 1995)
Rat hepatocarcinogenicity of MeIQx at low doses

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

**GST-P positive foci**

- 16 wks
- 32 wks

**MelQx-DNA adduct**

- *p<0.05
- 4 wks

**8-OHdG**

- *p<0.01
- 4 wks
**Big Blue Rat**

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

**in vitro packaging**

**Incubation containing X-gal**

**Phage**

**Liver**

**DNA isolation**

**Mutation in the lacI gene**

**lacI** **lacO** **αlacZ**

**Repressor**

**βgalactosidase**

**X-gal**

**Blue**

**In vivo mutagenicity test in Big Blue rats**

*(Plaque Color Screening Assay)*

MelQx

E.coli

Transcriptional

β-galactosidase

X-gal

Blue plaque = Mutation (+)

White plaque = Mutation (-)
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

* * p<0.01

lacI gene: 30~40 copies on chromosome 4 in the F344 rat
Frequencies of H-ras mutation and GST-P positive foci in the liver of rats treated with MeIQx

Detection of H-ras mutation:
Thermosequenase cycle end labeling (TCEL) method
Chemical carcinogenesis mechanism

- Carcinogen
  - Inactivation
  - Metabolic activation: ultimate carcinogen
  - DNA adduct formation
    - Oxidative stress
    - DNA repair error
    - Mutation: irreversible change
    - Cancer-irrelative mutations
    - Apoptosis
    - Preneoplasia: cell proliferation
    - Apoptosis
    - Cancer: malignancy

- Non-DNA
  - DNA repair

Initiation

Promotion

Carcinogen

Normal

Mutation

A→C mutation

http://www.intelihealth.com
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
MeIQx DNA adduct level and number of GST-P positive foci in the damaged liver of rats

Animals: 280 male F344 rats, 21-day-old

Thioacetamide
MelQx

* p < 0.01 vs 0 ppm

* p < 0.01 vs 0 ppm

* p < 0.01 vs 0.1 ppm

MeIQx-DNA adduct level and number of GST-P positive foci in the damaged liver of rats.
Risk of liver cancer: Response curves for the carcinogenicity markers dependent on the dose of MeIQx

Conclusion: Existence of a carcinogenic threshold, at least a practical threshold
Assessment of genotoxic carcinogens at low doses

Effects on various organs
  Liver, Colon, Kidney

Effects on various biomarker
  1. Carcinogen-DNA adduct
  2. In vivo mutagenicity
      Mutation frequency of lacI or gpt gene
  3. Oxidative DNA damage: 8-OHdG
  4. Preneoplastic lesion
      Liver: GST-P positive foci
      Kidney: atypical tubular hyperplasia
      Colon: Aberrant crypt foci (ACF)
  5. Tumor

Weights of evidence
Effects of IQ on development of GST-P positive foci and DNA adduct formation in livers of rats

Animal: 1,560 male F344 rats, 21-day-old

IQ : 0, 0.001, 0.01, 0.1, 1, 10, 100 ppm in diet

GST-P positive foci

<table>
<thead>
<tr>
<th>IQ (ppm)</th>
<th>No. of rat</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

IQ-DNA adduct

*p<0.001 v.s. 0.01 ppm

0, 0.001 ppm : under detection limit (<5x10^-10).
mRNA expression in liver of IQ-treated rats at week 16

* p<0.05 v.s. 0 ppm
PhIP

One of food-derived heterocyclic amines
Mutagenicity: positive
Carcinogenicity: colon
Daily intake: 0.005-0.3 µg/day
Rat colon carcinogenicity of PhIP at low doses: Aberrant crypt foci (ACF) and PhIP-DNA adducts

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

PhIP-DNA adduct (\$/10^8 ntd)

ACF (No./rats)

PhIP (ppm, in diet)

0 0.001 0.01 0.1 1 10 50 100 400

* p<0.01 v.s. 0 ppm

* * * * * * *

ACF in colon

PhIP-DNA adduct

ACF

1,920 male, 6-week-old, F344 rats

PhIP

Adduct level

16 weeks

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
PhIP carcinogenicity in azoxymethane-initiated rat colon carcinogenesis

Animals: 192 male, 6-week-old, F344 rats

Tumor: Adenoma + Carcinoma

PhIP in diet

Tumor incidence

PhIP dose (ppm)

Tumor multiplicity

PhIP dose (ppm)
N-Nitroso Compounds

- Air, water, and food, notably in nitrite-treated meat and fish products
- *in vivo* formation from nitrites or nitrates and secondary amines
- Diethylnitrosamine
- Dimethylnitrosamine
- Mutagen
- Hepatocarcinogen
- Daily intake: μg/day range level
Rat hepatocarcinogenicity of *N*-nitroso compounds: Induction of GST-P positive foci

**Diethylnitrosamine (DEN)**
- Male F344, 21-day-old, 1,957 rats
- **No. (No./cm²)**
  - *p* < 0.01 v.s. 0 ppm

**Dimethylnitrosamine (DMN)**
- Male F344, 21-day-old, 1,520 rats
- **No. (No./cm²)**
  - *p* < 0.01 v.s. 0 ppm
**LacI** mutation frequency and development of GST-P positive foci in the liver of Big Blue rats treated with DEN for 16 weeks

<table>
<thead>
<tr>
<th>DEN (ppm, in drinking water)</th>
<th>MF (No./10^6)</th>
<th>GST-P (No./cm^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.0001</td>
<td>5</td>
<td>1</td>
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<tr>
<td>0.001</td>
<td>10</td>
<td>2</td>
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<td>0.01</td>
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<td>3</td>
</tr>
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<td>0.1</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>150</td>
<td>5</td>
</tr>
</tbody>
</table>

*P < 0.05

AT→TA Transversion
AT→GC Transition

**AT→TA Transversion**

**AT→GC Transition**

*P < 0.05
Potassium bromate

Food additive
Contaminant in tap water

Genotoxicity
- Ames test: +
- Chromosome aberration test: +
- Micronucleus assay: +

Renal carcinogenicity in rats
\( \geq 250 \text{ ppm}: + \) (Kurokawa Y, 1983)
Mutation frequencies and oxidative DNA damage in kidney of Big Blue rats treated with potassium bromate

KBrO$_3$: potassium bromate

Total lacI mutation frequency

lacI mutation frequency (GC to TA)

Oxidative DNA damage (8-OHdG)

* p<0.05 vs 0 ppm
Promotion effects of KBrO$_3$ in kidney carcinogenesis induced by EHEN in Wistar rats

Animal: 240, 6-week-old, male Wistar rats

Groups 1-8 (30/group)

KBrO$_3$, 0, 0.02, 0.2, 2, 8, 30, 125, 500* ppm

Kidney: 8 slices/kidney (16 slices/rat)

Renal tumor

Atypical tubular hyperplasia

Number /rat

Number /area

Number /rat

Number /area

* p<0.001 v.s. 0 ppm

* p<0.0001 v.s. 0 ppm
Conclusions

Response curves for the effects of genotoxic carcinogens dependent on the dose

Carcinogenicity at high dose

Carcinogenicity at low dose

Existence of threshold (practical or perfect)
Recently, the concepts of “practical” and “perfect” thresholds for genotoxic carcinogens have been proposed. In these cases, activities of carcinogens are usually associated with a no-observed effect level (NOEL).

Thresholds in carcinogenicity
Genotoxic carcinogens and thresholds

1. Primary mutagenic carcinogen
   → Practical threshold
     : Heterocyclic amines, N-nitroso compounds

2. Secondary mutagenic carcinogen
   → Perfect threshold
     : Potassium bromate

3. Primary or secondary mutagenic carcinogen, but carcinogenicity based on cytotoxic mechanism
   → Perfect threshold
     : 1,4-Dioxane

4. Genotoxic, but non-mutagenic carcinogen
   → Perfect threshold
     : Dimethylarsinic acid
Since the threshold exists for genotoxic carcinogens, we should accept it for human risk assessment and management of environmental carcinogens, in particular for substances contained in food at low doses.
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