Mechanisms of Nongenotoxic Carcinogenesis

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Unequivocally Genotoxic Compounds, in the absence of other data, are presumed to be trans-species carcinogens, implying hazard for man

Need not be subjected to long-term carcinogenicity studies
Rodent Tumorigenesis vs. Human Carcinogenesis (?)

Chemicals / Pharmaceuticals Databases

US National Toxicology Program
Carcinogenic Potential Database
US FDA
CPMP (NL and GE)
Centre for Medicines Research
Japanese Pharm. Manufacturers Association
US Physician’s Desk Reference

Reopened (2010/11): Pharma/FDA/MHLW databases

+ Rodent tumorigens
~ 50% of compounds tested

IARC database
20 Human Carcinogens
few Non-genotoxic
Azathioprine
Cyclosporin A
Diethylstilbestrol
Dioxin

2011: Pioglitazone

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Getting the Supportive Data for Non-genotoxic Carcinogenesis

How and When?

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Supportive data for nongenotoxic carcinogenesis may be obtained through

- Collection of Specific Information Included in the Safety Assessment Protocols
- Separate Studies
ICH- S1B GUIDELINE

Repeated Dose Toxicity Studies May Provide Data on

- Metabolic disposition
- PK / TK
- Cellular / organ changes and reversibility
- Immunotoxicity

Biochemical Changes

- Hormonal
- Growth factors
- $\alpha_{2\mu}$-globulin

Drug-Receptor Interactions

DNA Adducts in the Target Cells
ICH Discussions
on
ICH-S1B GUIDELINE

Possible to Predict Epigenetic Carcinogenesis as the Outcome of 2Y Rodent Studies?

Eg Using WOE approaches?
Main Mechanisms of Non-genotoxic Carcinogenesis
The Multistage Theory of Carcinogenesis

**Initiation**
- DNA damage

**Promotion**
- Cell proliferation (growth)
  - Cytotoxicity
  - Drug-receptor interaction
  - Modulation of signal transducing pathways
  - ↓ GJ intercellular communication
  - ↓ Apoptosis
  - ↓ Tumour suppressor gene function

**Epigenetic**
- Modified regulation of growth factors receptors
- tRNA
- Cell cycle -regulation proteins

Direct interaction
Nongenotoxic Carcinogenesis

Indirect Regulation of DNA Expression
(possibly heritable)

Genetically Unstable Cells

Malignant Cell Transformation
Main Characteristics of Non-genotoxic Carcinogenesis

- **Specificity**
  - Species
  - Sex
  - Organ

- **Threshold required for**
  - Cell proliferation
  - Tumour development

- **Stepwise dose-response curve**
  - Exposure vs. Cell proliferation
  - Cell proliferation vs. tumour development

- **Reversibility**
Mechanisms of Non-genotoxic Carcinogenesis

Cytotoxicity

↑ Urine pH
Silicate Microcristals

Sodium Saccharin

Urinary Bladder Tumours

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Mechanisms of Non-genotoxic Carcinogenesis

Cytotoxicity

↑ Urine pH
Silicate Microcristals

Acid Saccharin

No Urinary Bladder Tumours

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Chronic Cell Injury

$\alpha_2\mu$ globulin

limonene

Proximal tubular cells

Kidney neoplasia

Male Rat

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Chronic Cell Injury

\[ \alpha_2\mu \text{globulin} \]

Limonene

Kidney neoplasia

Male Rat

tubular cell death

cell proliferation

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Hormone-Induced Tumorigenesis

THYROID

Possible Mechanisms

- **Intrathyroidal mechanisms** *(thioureylenes, sulphonamides)*
  - Iodine uptake
  - Hormone synthesis

- **Extrathyroidal Mechanisms** *(liver enzyme inducers)*
  - Hormone metabolism
  - Hormone disposition
Hormone-Induced Tumorigenesis

Thyroid Tumour (rodents)

Hypothalamus → Pituitary → TSH → Follicular Cell

AC → cAMP → ↑ Cell Proliferation

↓ T₃, T₄

↓ Thyroxine Metabolism

Hormone Synthesis

Adenomas
Carcinomas

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## Thyroid Gland Biochemistry / Physiology

### Species Differences

<table>
<thead>
<tr>
<th></th>
<th>Rodents</th>
<th>Primates</th>
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<tbody>
<tr>
<td>TBG</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>$T_4$ half-life</td>
<td>12 Hours</td>
<td>5-6 Days</td>
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<tr>
<td>Spontaneous Thyroid Follicular Cell Neoplasia</td>
<td>2%</td>
<td>0.004%</td>
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<tr>
<td>TSH ratio</td>
<td>25</td>
<td>1</td>
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Drug-induced Thyroid Tumorigenesis (Rodents)

Risk Assessment

Possible Species Specificity (Rodents)

Confirmatory studies

- Thyroid function evaluation
- Correlation with cell proliferation
- Reversibility after drug withdrawal

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Sulfamethazine

Inhibition of Thyroid Gland Microssomal Peroxidase

Thyroid Neoplasia

Percent Decrease

Log Dose (ppm)

T₃

Percent Decrease

Log Dose (ppm)

T₄

Percent Decrease

Log Dose (ppm)

TSH

Percent Decrease

Log Dose (ppm)

Thyroid Weight

% of Body Weight x 1000

Log Dose (ppm)
Steroid Hormone-Induced Tumorigenesis

Main mechanisms

✓ Direct Interaction With Steroid Receptor

✓ Drug-Mediated Increase of Steroid Hormones, eg
  
  • Nitrofurantoin-Induced Mice Ovary Carcinogenesis
  
  • Dopamine Antagonists Induced Carcinogenesis
Steroid Hormone-Induced Tumorigenesis

Nitrofurantoin

Destruction of Graafian Follicles

Hypothalamus

↑ GnRH

↓ Estrogens

Pituitary

↑ LH

↑ FSH

Ovary tumours

Ovary

Nitrofurantoin (B6C3F1mice; NTP)

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Steroid Hormone-Induced Tumorigenesis

Hypothalamus

Dopamine Antagonists

Estrogens

Pituitary

↑ Prolactin

Ovary

↑ Corpora lutea

↑ Progestagens

Mammary gland tumours

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HORMONE-INDUCED TUMORIGENESIS

Disturbance of Gastric Acidity

Mechanism Relevant for Man

Further Risk Assessment

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The Signal Transduction Model of Carcinogenesis

PP clofibrate rat

Gene expression... specific enzymes (acyl CoA oxidase)
Peroxis. Prolifer.
Liver Tumour

AH dioxin rat, human

gene exp. → CYP450; CYP1A1; CYP1A2
TK → UGT-1 → T₄ clearance
Thyroid Tumour

βadrenoceptor stimulation (mice) → ↑ cAMP
Uterus Mioma
Ovarium
Leyomioma

Drug → R
Gs → cAMP → cAMP-PK
Gp → ↑ IP₃+DAG → PKC
cfos cmyc → Cell Proliferation

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Receptor Mediated carcinogenesis: Recent Cases

**Drug** → **R** → **Gs** → **cAMP** → **cAMP-PK** → **cfos cmyc** → **Cell Proliferation**

- **PPARγ**
- **rat**
- **Urothelial Hyperplasia (3 Months)** → **R** → **Bladder tumors**

- **GLP1R Agon.** → **R** → **Insulin secretion** → **Thyroid C Cell Tumours**

- **SGLT1 antagonists** → **R** → **Ca??** → **Tumors Renal Leydig C. / adrenals**
PIOGLITAZONE and Bladder Cancer

Nonclinical Background
Carcinogenic Potential of Pioglitazone

Non Genotoxic

RAT TOXICOLOGY:

Repeated Dose Studies:

Increased urothelial hyperplasia in males and in females (at 63mg/Kg)

2Y Carcinogenicity:

-urothelial transitional cell adenoma/carcinoma in males only (from 4mg/Kg).
Applicant’s proposed Mechanism
The Cristal Theory of PIO-induced tumorigenesis

Cytotoxicity

↑ Urine pH
↑ Microcristals

PIO?

Urinary Bladder Tumours

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CHMP Position

• 1st mechanistic study:
  – Only 60% correlation between urinary crystals and tumors was observed.
  – Although crystals may be involved, a PPAR-gama-mediated promoting effect cannot be excluded.
  – Human relevance to be further addressed.
  – (PPARgamma presence in the bladder not known yet)
PPAR – Mediated Effects

• Cell proliferation promoting Effects

• Apoptosis Modulatory Effects

Influencing:
  adipocyte differentiation (multiple receptors involved; Through stem cells??)

TUMORIGENIC / ANTITUMORIGENIC??
Study Outcome

• **NH4CL reduced crystals:**
  Group 1 and 3 (diet): higher level of microcrystals than Group 2 and 4 (diet with NH4CL) irrespective of PIO.

• **Histopathology**
  – hyperplastic changes not correspondent to microcrystals
  – hyperplastic changes (simple, nodular and papillary) only significantly occurred with PIO. irrespective of diet or level of microcrystal formation.
  – Animals with PIO + NH4Cl had reduced microcrystal formation and lower severity of the histopathological findings
Study Outcome

Conclusion

Results indicates that microcrystals are not responsible for the observed hyperplastic changes.

• The presence of microcrystals may exacerbate the response BUT ARE NOT considered to be the cause of the hyperplastic changes.

• The “crystal hypothesis” to explain the carcinogenic bladder cancer findings in the original carcinogenicity study in rats administered Pioglitazone is contradicted.

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If reversibility of effects (such as hyperplasia) was demonstrated in repeated dose toxicity studies that included a drug free period it may provide reassurance that the findings will not be irreversible.

If good mechanistic data for carcinogenic effects are available, it may help in the assessment of relevance to human safety.

The applicant should be encouraged to develop and identify relevant and valid biomarkers that may be clinically monitored.

Compounds that result in tumours in more than one species and at more than one site, without adequate safety margins, may contribute to an increased level of concern.
Questions and answers on the review of pioglitazone-containing medicines (Actos, Glustin, Competact, Glubrava and Tandemact)

Outcome of a procedure under Article 20 of Regulation (EC) No 726/2004

The European Medicines Agency has completed a review of pioglitazone-containing medicines, following concerns over the possible risk of bladder cancer. The Agency’s Committee for Medicinal Products for Human Use (CHMP) concluded that, although there is a small risk of bladder cancer with pioglitazone, this is outweighed by the benefits for the patient.
European Medicines Agency recommends new contra-indications and warnings for pioglitazone to reduce small increased risk of bladder cancer

Benefit-risk balance remains positive in a limited population of type 2 diabetics

Finalising its review on antidiabetic pioglitazone-containing medicines and the occurrence of bladder cancer, the European Medicines Agency’s Committee for Medicinal Products for Human Use (CHMP) confirmed that these medicines remain a valid treatment option for certain patients with type 2 diabetes but that there is a small increased risk of bladder cancer in patients taking these medicines.

However, the CHMP also concluded that the small increased risk could be reduced by appropriate patient selection and exclusion, including a requirement for periodic review of the efficacy and safety of the individual patient’s treatment.

Prescribers are advised not to use these medicines in patients with current or a history of bladder cancer or in patients with uninvestigated macroscopic haematuria.

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Assays to Predict / Support Non-genotoxic Carcinogenesis

✓ **Short Term Assays**

*in vivo*
- Initiator / promotor assays (1 or several initiators)
- Transgenic species?

*in vitro*
- Cell transformation assays (SHE/SA7; balbc3t3)
- Hepatic cell proliferation (SDS)
- BrdU labelling
- Metabolic cooperation (V79)
- PKC activation

✓ **Mechanistic Studies**

✓ **Genomics / Biomarkers**
Gap Junction Intercellular Communication

(GJIC)
Connexins and GJIC

Most are phosphoproteins regulated by TK and oncogenes.

Function modified by serine/threonine phosphorylation pattern.

Correlated with altered GJIC.
CONCLUSION

Human Risk Assessment of Positive Findings
Risk Assessment of Rodent Tumorigens

Mechanistic Data

Level of Relevance for Man

- Relevant
- Not Relevant
  - No Risk

Dose-Response Curves

- Benefit/Risk
- RMP & SPC

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Thank You

The END

END