Paternal carcinogen exposures and genetic risk in their offspring

Proposal for the CEFIC-LRI Innovative Science Award

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Benzo[a]pyrene

1930 Kennaway & Cook
benzo[a]pyrene isolated from coal tar
1933 Yamagiwa & Ichikawa
benzo[a]pyrene is carcinogenic in rabbits

Ubiquitous environmental & occupational pollutant

Over 70 years of research on Benzo[a]pyrene

What is missing?
Germ-line mutations

Role of B[a]P-DNA adducts in inducing mutations in somatic cells is undisputed:

......but their role in inducing germ-line mutations is not thoroughly investigated:

• No human studies
• Limited research in experimental animals

OBJECTIVE 1

1) Impact of DNA damage in gametes induced by paternal low dose exposure to benzo(a)pyrene on the formation of germ line mutations
Protective mechanisms

Germ cells can be protected against parental exposures to carcinogens, via a complex network of molecular mechanisms:

- DNA repair: Removal of damage
- P53: Provide time for repair/apoptosis
- Heat Shock Proteins: Essential for gametogenesis

To study the role of protective mechanisms; Knock-out DNA-repair

**OBJECTIVES 2 & 3**

2) Does modulation of DNA repair affect germ line mutagenesis?

3) To investigate potential protective mechanisms of sperm against exposures to benzo(a)pyrene
Overall study design

Paternal exposure to B[a]P (and unexposed controls) is followed by crossing these animals as follows:

<table>
<thead>
<tr>
<th>+B[a]P males:</th>
<th>Wt</th>
<th>XPA(-/-)</th>
<th>XPA(-/-)</th>
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<table>
<thead>
<tr>
<th>-B[a]P females:</th>
<th>Wt</th>
<th>Wt</th>
<th>XPA(-/-)</th>
</tr>
</thead>
</table>

Crossing at several time points after exposure:

**Stemcells → Mature sperm**
Methods

Assessment of following parameters:

DNA damage in germ cells of male mice

minisatellite mutations in offspring

B[a]P-DNA adducts by $^{32}$P-postlabeling

Transcriptomics

changes in gene-expression profiles to elucidate potential protective mechanisms in testis
Variable Number Tandem Repeats

- in nuclear genome highly repeated DNA sequences; tandem repeats
- mostly transcriptionally inactive
- non-coding DNA
- high rate of mutation
- mutation rate correlates with rate at coding loci

0.1-20 kb long, 6 repeat units
Proof of concept......

- Herring gulls


- Mice


32P-postlabeling

- Currently most sensitive assay for the detection of B[a]P - DNA adducts
- Routinely applied in our laboratory

\[
\text{DNA} 
\]

\[ \text{MN/SPD digestion} \]

\[ \text{Np + Xp} \]

\[ \text{Nuclease P1} \]

\[ \text{Butanol extraction} \]

\[ \text{N + Xp} \]

\[ \text{T4 kinase} \]

\[ [\gamma - 32P] \text{ ATP} \]

\[ \text{Xp} \]

\[ *\text{pXp} \]

\[ *\text{pXp} \]

\[ \text{TLC} \]

\[ \text{Phosphorimaging} \]

\[ \text{Lung-DNA smoker} \]

\[ \text{BPDE-DNA} \]

\[ \text{Maps of 32P-postlabelled adducts} \]
Transcriptomics

1. Selfmade mouse microarray
   Based on the PHASE I human microarray, containing >600 genes

2. Toxicologically relevant,
   Several pathways, including:
   - Inflammation
   - DNA damage & repair
   - Oxidative stress
   - cell-proliferation / apoptosis

3. Validation by RT-PCR

Identification of new protective pathways?
Reaching the objectives

**Objective 1**  Role of B[a]P in germ line mutagenesis

- Comparing mutation frequencies in offspring of exposed males with offspring of unexposed controls.
- DNA adduct levels in testis (Biologically Effective Dose)

**Objective 2**  Role of DNA repair in germ line mutagenesis

- Comparing mutation frequencies in offspring of exposed XPA-/- males with offspring of exposed wildtype controls.
- DNA adduct levels in testis

**Objective 3**  Protective mechanisms

- Comparing gene-expression in offspring of exposed XPA-/- males with offspring of exposed wildtype controls.
- DNA adduct levels in testis
**Timeframe**

0 - 4 months
Experimental conditions will be optimised before the actual breeding experiment starts

4 - 14 months
Main breeding experiment and collection of tissues

9 – 20 months
Analysis of samples

20-24 months
Data collection and statistical analysis
Writing of report & scientific publications
Dissemination

• Publications in scientific journals
• Presentations at scientific meetings
• Special website created for this proposal
• Communication to CEFIC
Benefit for CEFIC, Science & Society

- Further completion of the B[a]P-puzzle
- Towards a future with reduced animal usage for toxicity testing?
- Improved knowledge → Better protection of workers
Project Research Team

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Collaborations

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