Inhibition of DNA double strand break repair by uranium and links with effects on reproduction of the zebrafish

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Uranium

nuclear fuel cycle, military, industrial & agricultural applications

Contamination of aquatic ecosystems

Background [U] surface water: ~ ng/L to 6 µg/L

Vicinity of former uranium mines: ~ tens - 100 µg/L

U drinking water guideline: 15 µg/L WHO

Environmental Quality Standards range from 1 µg/L to 100 µg/L
### Objective

- Assess genotoxicity markers as predictive biomarkers for U effects on individuals or populations.

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**Biological endpoints**

<table>
<thead>
<tr>
<th>Ecosystem</th>
<th>Ecological relevance</th>
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<tr>
<td>Population</td>
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<td>Organism</td>
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<td>Tissue</td>
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<td>Cell</td>
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<td>Molecule</td>
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**Genotoxicity**

- DNA is the 1st cellular target for radionuclides.
  - DNA damages in:
    - Somatic cells: carcinogenesis
    - Germ cells: teratogenesis and reprotoxicity

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**Mechanisms**

- DNA damages in somatic cells lead to carcinogenesis.
- DNA damages in germ cells lead to teratogenesis and reprotoxicity.
Zebrafish

- Easy breeding (mature in 3 month)
- Fully sequenced genome
- Model for developmental studies
Significant increase of DNA damage in males and females gonads
Decrease of fecundity & of egg and larvae viability
DNA damages are still observed after 28 days of depuration → occurrence of DNA Double Strand breaks (DSBs) ? correlation with the decrease of fertility ?
Does U induce DNA DSBs in zebrafish?

Cells in G0/G1

DNA Double Strand Breaks

DSB repair

Time-response Experiments

DNA repair kinetics
Number of foci at 10min, 1h, 2h, 4h, 24h

NHEJ repair Pathway

Ku70, Ku80

DNApK

DSB detection

Dose-response experiments

H2AX

ATM

MRE11 dependant recombination Pathway

Cytogenetic abnormalities

Micronuclei number

C. Adam, IRPA13, Glasgow, May 16th, 2012
Uranium induces DSB in ZF4 cells

- Increase of the nb of γ-H2AX foci per cell up to 100 µM

- No cytotoxicity up to 500 µM

Pereira et al, Aquatic Toxicology, 2011
Occurrence of SSBs concomitant to DBSs formation

Alkaline comet assay

Pereira et al, Aquatic Toxicology, 2011
Genotoxicity of uranium on ZF4 cells

Increasing of U precipitates with the decrease of DSBs

Pereira et al, Aquatic Toxicology, 2011
Genotoxicity of uranium on ZF4 cells

Urchins-like thin needle-shaped uranium structures, mainly concentrated in lyzosome-like vesicles.
NHEJ repair was disrupted in uranium contaminated ZF4 cells: no DNApK foci was observed after 24 h of 10 µM uranium exposure

Pereira et al, Aquatic Toxicology, 2011
Conclusions

✓ Uranium induces DNA SSBs, DSBs and micronuclei

✓ These damages are probably linked to U reprotoxicity (similar internal [U] concentrations for in vivo & in vitro studies)

✓ Uranium detoxication in lyzosome-like structures may explain the low cytotoxicity (< 10 %)

✓ Inhibition of NHEJ (DNA PK) complemented by another non specific DNA repair mechanism ?
Thank you for your attention

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