Genotoxicity of Doxorubicin in F344 Rats by Combining the Comet Assay, Peripheral Blood Micronucleus Test, and Pathway-Focused Gene Expression Profiling

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FDA/NCTR/DGMT
DISCLAIMER

This work was supported by the US Food and Drug Administration. The views presented here are not necessarily endorsed by the US FDA.
Outline

- DOX
- COMET ASSAY
- EXPT DETAILS
- RESULTS
- CONCLUSIONS
DOX CHEMICAL STRUCTURE
DOX

- DOX is an anthracycline (ANT) antibiotic effective against leukemia, lymphoma, and other solid tumors.

- In pediatric oncology, ANT's increased 5-year survival rate for childhood cancer from about 30% in 1960s to 80% today.
DOX

- The risk of cardiotoxicity is the most serious drawback to the clinical efficacy of DOX

- Antitumor activity of DOX is likely to be distinct from cardiotoxicity

- Oxidative stress involving intramyocardial production of ROS is widely accepted mechanism for cardiotoxicity
Questions

- Is DOX genotoxic to heart?

- Does it induce DNA damage/oxidative DNA damage?
DNA Damage and Consequences

DNA damage caused by genotoxic agents is repaired, but if not repaired or erroneously repaired, it may, together with cellular response, induce genomic instability culminating in disease processes via multiple pathways:

- gene mutations
- altered DNA repair capacity
- chromosomal aberrations
- clonal heterogeneity
- cellular transformation, etc.
Implications of DNA Damage in Diseases

DNA damage
(erroneously repaired/ unrepaired)

Somatic cells
- Aging
- Cancer
- Neuro-degenerative diseases

Germ cells
- Sterility

Cardiovascular diseases
- Hepatitis
- Birth defects
  - Cystic fibrosis

Metabolic disorders
- Diabetes
- Hematological disorders
- Systemic erythematosis

Neuro-degenerative diseases
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Cardiovascular diseases
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Somatic cells
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Types of DNA Damage Detected in the Comet Assay

1. DNA double-strand breaks
2. DNA-DNA and DNA-protein cross links
3. Single-strand breaks and/or strand breaks induced by alkali-labile sites (AP sites that are potentially mutagenic)
4. Oxidized bases (oxidative DNA damage)
Detection of Oxidative damages

The incorporation of extra step of digestion with lesion-specific endonuclease (e.g., Fpg - formamidopyrimidine glycosylase, endonuclease III, uracil glycosylase) following lysis allows quantitative detection of specific kinds of DNA damage such as oxidative DNA damage which is implicated in several health conditions.

- The DNA repair enzymes recognize oxidized purines and pyrimidines, and convert them into DNA strand breaks that increase comet migration.
## DOX IV Treatment Schedule

<table>
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<tr>
<th>Time</th>
<th>1st dose</th>
<th>2nd dose</th>
<th>3rd dose</th>
<th>4th dose</th>
<th>Sacrifice</th>
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<tr>
<td>0 hr</td>
<td>DOX</td>
<td>DOX</td>
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<tr>
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<td>CP</td>
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<td>10 mg/kg</td>
<td>10 mg/kg</td>
<td>100 mg/kg</td>
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</table>
Comet Assay/ Glycosylase Enzymes

Cells mixed with agarose are added to a slide.

Cells are lysed in a high salt solution to remove the cell membrane and leave the nuclear DNA.

Slides are immersed in alkaline (pH>13) buffer and denatured allowing expression of strand breaks.

Current is applied to the slides and the smaller pieces of DNA migrate away from the nucleus.

DNA migration is measured by image analysis.

Fpg, ENDO III, hOGG1
RT² Profiler PCR Arrays
(pathway-focused DNA damage and repair genes)
RESULTS
Focal infiltrates of a few macrophages and lymphocytes in the myocardium in both untreated and DOX treated F344 rats (arrows)
DOX–induced DNA damage
DOX-induced DNA Damage
Fpg

Heart

Liver

[Bar graphs showing DNA damage in Heart and Liver with DOX treatment]

* indicates statistical significance
DOX-induced DNA damage
Endo III

Heart

Liver
DOX-induced DNA Damage

hOgg1

Heart

Liver

[DOX (mg/kg)]

% DNA in Tail

*
Pathway-focused array analysis of DNA damage and repair genes in the heart of DOX-treated rat
CONCLUSIONS

- DNA damage via strand breaks was not the primary mechanism of DOX-induced genotoxicity in F344 rats
- Dox induced dose-dependent increase in the oxidative DNA damage in the cardiac tissue
- Dox induced significant expression of 11 genes related to apoptosis, cell-cycle checkpoints, damaged DNA binding, BER, DSBR
Research Article

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THANK YOU