RADIATION-INDUCED DAMAGE TO DNA: ASSESSMENT OF OXIDATIVELY GENERATED LESIONS

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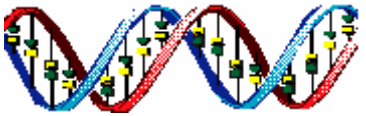
OXIDATIVELY GENERATED DAMAGE TO DNA

PHOTOSENSITIZATION — IONIZING RADIATION UV LASER PULSES — OXIDATIVE METABOLISM XENOBIOTICS

$^1O_2$ — $- e^-$ — $\cdot OH$ ← H_2O_2 ← $O_2^{\cdot -}$

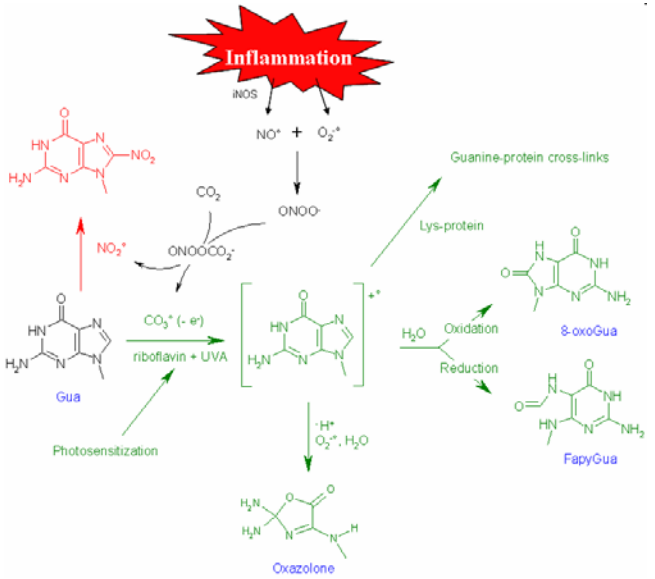


- Modified bases
- Abasic sites
- Single & double strand breaks
- DNA-protein cross-links
- Aldehyde adducts to aminobases

LETHALITY — MUTAGENESIS — CARCINOGENESIS — AGING

REACTIVE OXYGEN AND NITROGEN SPECIES *(reactivity)*

- Superoxide (hydroperoxide) radical: *no detectable reactivity toward DNA*
- Hydrogen peroxide: *low reactivity with adenine and implication in Fenton reaction*
- ·OH radical: *reacts with all bases and the sugar moiety*
- Singlet oxygen ($^1O_2$): *[4+2]-cycloaddition to guanine*
- Ozone (O_3): *only reacts with pyrimidine bases*
- HOCl: *halogenation of purine and pyrimidine bases*
- Peroxynitrite (ONOO-): *reacts with guanine*
- One-electron oxidizing agents: *hydration & deprotonation of base radical cations*



(Cadet *et al*, *Nature Chem Biol*, 2006)

Measurement of oxidatively generated damage in cellular DNA
(outline)

- Measurement of oxidatively damaged DNA

  HPLC-MS/MS

  Modified comet assay

- Damaging agents

  Ionizing radiation (OH radical)

  UVA radiation (singlet oxygen)

OXIDATIVELY GENERATED BASE DAMAGE TO CELLULAR DNA
(current situation)

* Isolated DNA and model compounds:

  More than 80 lesions have been identified as oxidative degradation products of thymine, cytosine, adenine, guanine and 5-methylcytosine

* Cellular DNA: only 14 base lesions have been accurately measured:

  Adenine (2)

  Guanine (2)

  Thymine (6)

  Cytosine (4) *(clustered damage)*

MEASUREMENT OF OXIDIZED DNA BASES
(*aims*)

- Search for markers of exposure to oxidative stress
  (delineation of the specificity of a given agent)

- Insights into the mechanism of formation
  (chemistry in the cell)

- Kinetics of repair within cells
  (decrease in the level of damage with time following exposure to oxidizing agents)

MEASUREMENT OF OXIDATIVELY GENERATED BASE DAMAGE TO DNA

- As DNA fragments
  (bases, nucleosides or nucleotides)

- In whole DNA

- In intact cells

MEASUREMENT OF BASE DAMAGE TO CELLULAR DNA
(*individual measurement*)

- Hampered by drawbacks until recently:

  Artefactual oxidation (derivatization of GC/assay)

  Spurious oxidation during DNA extraction

  (this has led to overestimation of values up to 3 orders of magnitude)

- Accurate chromatographic assay:

  HPLC-MS/MS

  Optimization of DNA extraction conditions

HPLC-MS/MS measurements of oxidized bases and nucleosides

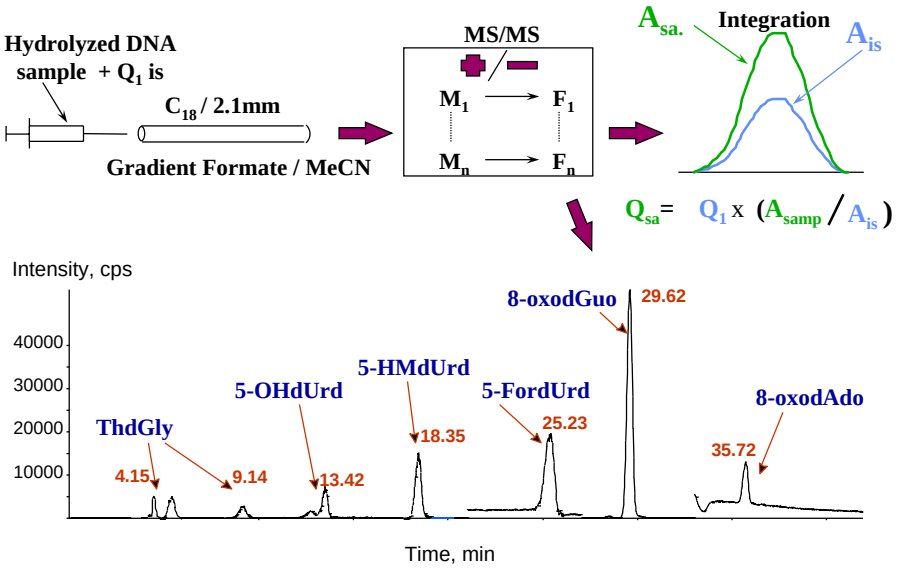
- Most recent method *(tandem mass spectrometers)*

- More sensitive than any other chromatographic methods by about a factor of 10 *(this depends on the targeted lesion)*

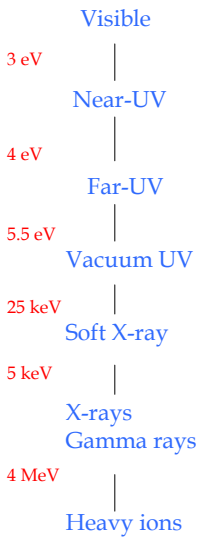
- More straightforward than GC-MS *(no derivatization)* and versatile than HPLC-ECD *(almost all compounds can be detected)*

- Extension to more sensitive analytical methods *(micro-HPLC, capillary electrophoresis)*

HPLCMS/MS of oxidized nucleosides
(separation - detection – quantitation)



Effects of environmental radiations on DNA



Photosensitization: one-electron oxidation, $^1O_2$

Excitation of the bases: dimerization, adducts

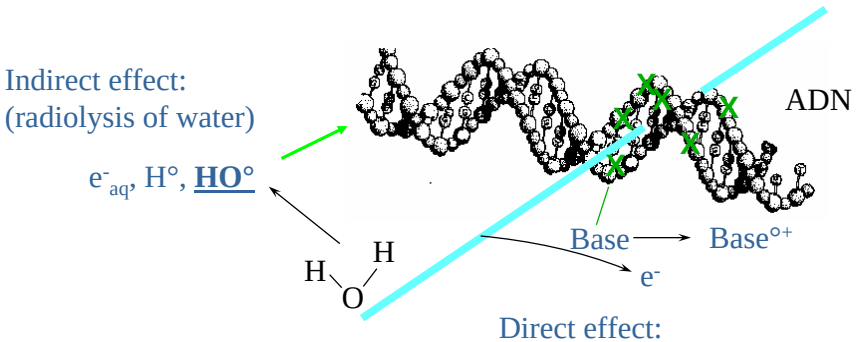
Photoionization: base, 2-deoxyribose

Atom photoexcitation: Auger effect (P = 2.153 keV)

DNA ionization: direct effect (radical cations)
Water radiolysis: indirect effect (·OH radical, e^-_{aq})

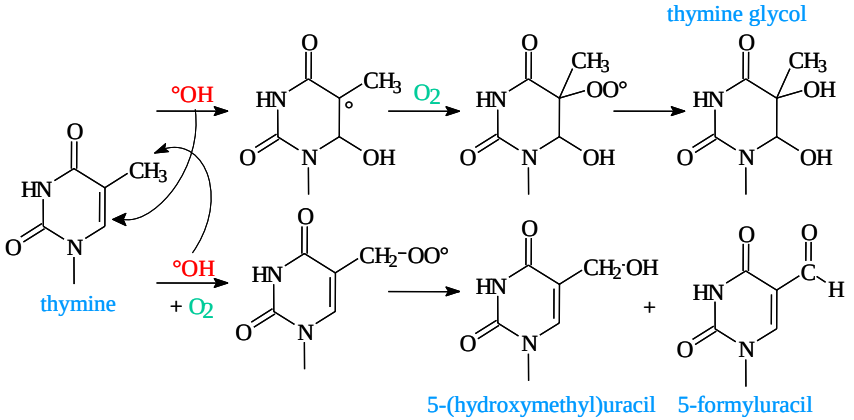
Multiple events: clustered damage

Radiation-induced damage to DNA



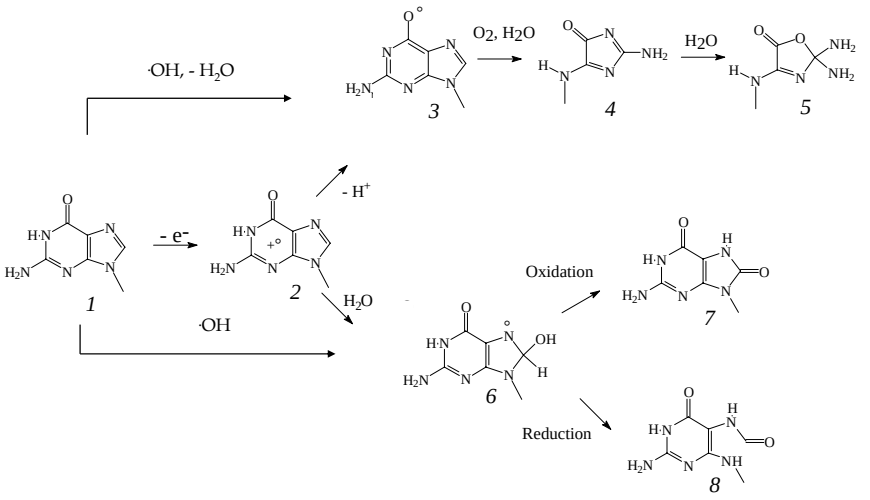
Aims of the work:
- identification of the lesions (biomarkers)
- measurement of DNA damage in cells

MAIN OXIDATIVELY GENERATED THYMIDINE DAMAGE IN CELLULAR DNA



(Cadet et al, 2005; 2007; Douki et al, 2006)

OH radical reactions of the guanine moiety



Kasai *et al*, JACS 1992; Cadet *et al*, JACS 1994; Gasparutto *et al*, JACS 1998, Ravanat *et al*, JACS 2003

Molecular effects of the UV components of solar radiation on cellular DNA

- UVB radiation

  oxygen independent photoreactions

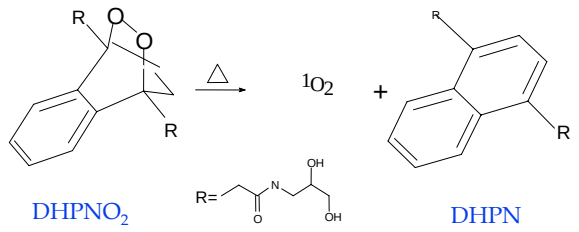
  targets: mostly bipyrimidine sites

- UVA radiation

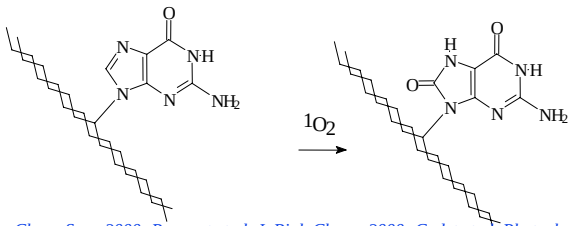
  photosensitization reactions (partly oxygen independent)

  photodynamic effects: guanine = main target (singlet oxygen)

Singlet oxygen oxidation of cellular DNA

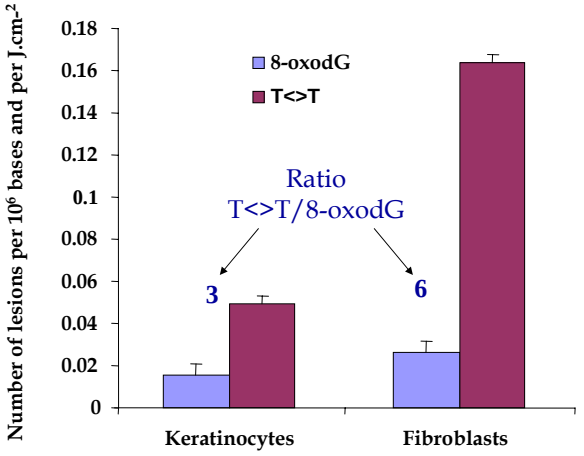


$DHPNO_2$ DHPN



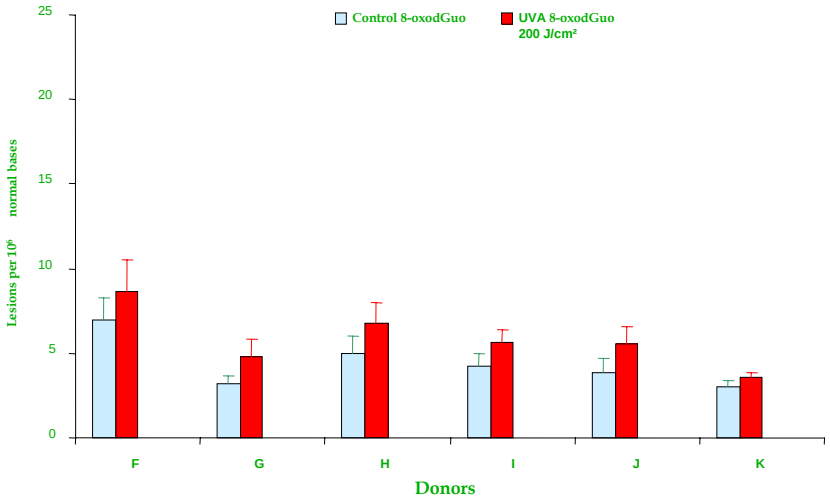
(Martinez et al, J. Am. Chem. Soc., 2000; Ravanat et al, J. Biol. Chem., 2000; Cadet et al, Photochem.Photobiol., 2006)

UVA-induced DNA damage in keratinocytes and fibroblasts



(Courdavault et al, Mutat Res, 2004)

Formation of 8-oxodGuo in the DNA of human skin upon exposure to UVA radiation



Mouret *et al*, *Proc. Natl. Acad. Sci. USA*, 103 (2006) 13765-70

MEASUREMENT OF LOW LEVELS OF RADIATION-INDUCED DAMAGE TO CELLULAR DNA

- Modified comet assay *(use of DNA repair enzymes to convert base damage into strand breaks)*
- Measurement of classes of damage *(less informative than HPLC-MS/MS)*
- High sensitivity *(almost complete lack of artefactual background allowing detection of damage at doses at low as 0.2 Gy)*

Comet assay associated with DNA glycosylases *(Fpg)*

Cells

Alkaline cell lysis

SSB + DSB + SAL

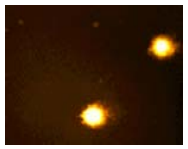
Treatment with Fpg

electrophoresis

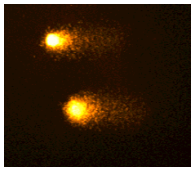
SSB + DSB + SAL + Fpg sites

Analysis

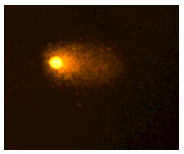
electrophoresis



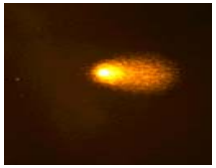
Control cells



Control cells treated wiht Fpg

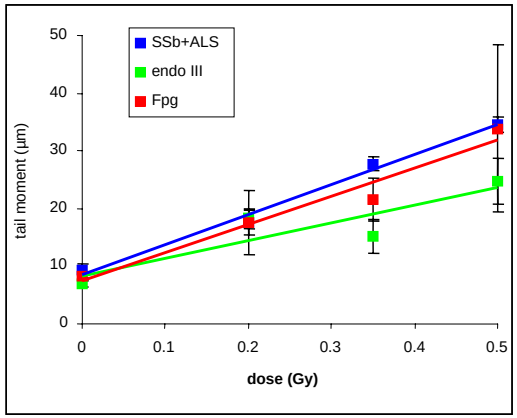


8 Gy irradiated cell



8 Gy irradiated treated with Fpg

Detection of radiation-induced DNA damage using an optimized version of the comet assay



SSB: strand breaks

ALS: alkali-labile sites

Endo III sites: oxidized pyrimidines

Fpg sites: oxidized purines

Cultured human monocytes exposed to γ-rays

(Sauvaigo et 2002)

OXIDATIVE GENERATED DAMAGE TO CELLULAR DNA *(conclusions)*

- *The steady-state level of the main oxidized bases is within the range of 1 lesion per 10^6 - 10^7 normal bases.*
- *HPLC-MS/MS and HPLC-ECD are operative for measuring acute effects of strong oxidizing agents and for level of DNA > 30 µg .*
- *Enzymic assays are appropriate for low amounts of DNA and to deal with slights variations in the level of oxidized bases (typically chronic exposure, antioxidants studies).*
- *Still a paucity of information on several lesions (secondary oxidation products, tandem modifications, DNA-protein cross-links)*
- *Aldehyde-aminobase adducts (oxidation of the 2-deoxyribose moiety)*

OXIDATIVELY GENERATED DAMAGE TO CELLULAR DNA *(ESCODD)*

- *"European Standards Committee on Oxidative DNA Damage": set up in 1997 with EC funding over the period 2000-2003; it has involved 25 member laboratories in Europe and one in Japan*
- *Objectives: to standardize and validate procedures for measuring 8-oxodGuo as a biomarker of DNA oxidation*
- *Levels of 8-oxodGuo (1.0) and Fpg-sensitive sites (0.1) per 10^6 bases in the DNA of human lymphocytes by HPLC and enzymic methods*
- *It will be necessary to re-examine anti-oxidants studies that are based on claims of much higher values of 8-oxodGuo than 1 per 10^6 bases*

(Collins et al, *Free Radic. Biol. Med.*, 2003; *Arch. Biochem. Biophys*, 2004, *FASEB J.*, 2005)

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Recent and current European research projects devoted to damage to cellular DNA

- ESCODD (EU network, 2000 - 03) *"European Standards Committee on Oxidative DNA Damage"*

- CLUSTOXDNA (EU Research Training Network, 2004 -07) *"Clustered oxidative damage to DNA: formation and biological features"*

- COST ACTION P9 (EU network, 2004 - 07) *"Radiation damage in biomolecular systems"*

- ESA (European Space Agency, 2003 -) *"Effects of space radiation on DNA within resistant cells"*

- CEFIC (European consortium of chemical industries, 2004 -): *"Effects of alkylating agents on DNA"*

- COST ACTION CM0603 (EU network, 2007 - 10) *"Free Radicals in Chemical Biology"*