Redox homeostasis & oxidative stress

Redox homeostasis
- natural levels of oxidants (O_2) and antioxidants in each cell

Disruption of redox homeostasis

-> depletion of oxygen: metabolism disruption, acidosis in tissues, cell necrosis

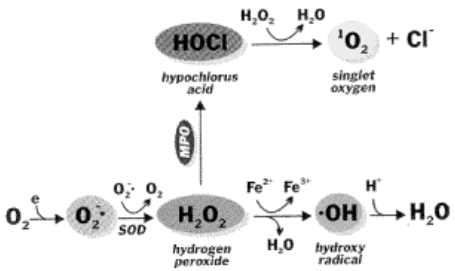
-> overproduction of oxidants: depletion of antioxidants, oxidation of biological molecules (membranes, proteins, DNA ...)
-> disruption of signalls (GSH), carcinogenesis, health problems, necrosis ...
= oxidative stress

Overproduction of oxidants

Oxygen – principal molecule in living organisms
Oxygen increase or reactive derivatives -> toxicity

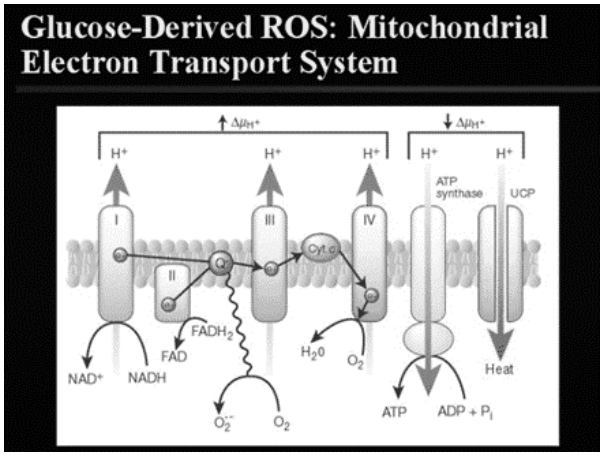
ROS = Reactive Oxygen Species: *Sources*
- production in mitochondria (byproducts)
- redox-cycling (quinones of xenobiotics)
- Fenton-reaction (metals)
- oxidations mediated via MFO (CYP)
- depletion of antioxidants (reactive molecules)

Reactive Oxygen Species (ROS)

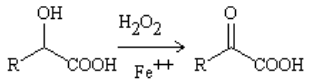


Druh	Symbol	Poločas života (s) při 37 °C
Superoxid	$O_2^{\bullet -}$	1×10^{-6}
Hydroxylový radikál	$OH^{\bullet}$	1×10^{-9}
Alkoxylový radikál	$RO^{\bullet}$	1×10^{-6}
Peroxylový radikál	$ROO^{\bullet}$	1×10^{-2}
Singletový kyslík	O_2	1×10^{-6}
Molekulární kyslík	O_2	$> 10^{2}$

ROS & mitochondria

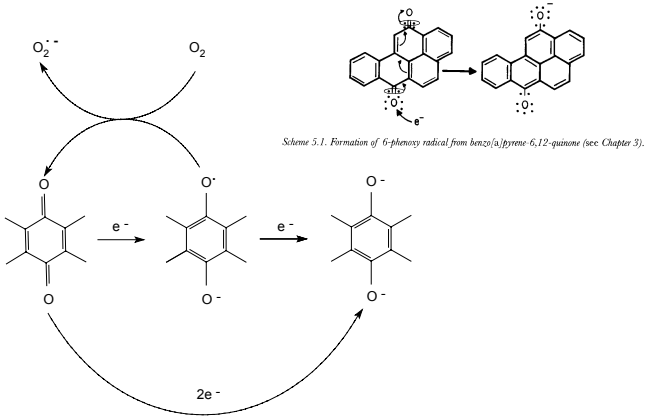


Fenton reaction



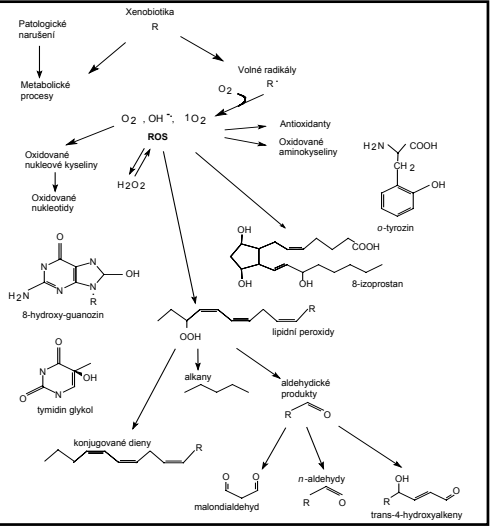
$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH + OH^-$$

Redox-cycling and ROS formation



Scheme 5.1. Formation of 6-phenoxy radical from benzo[a]pyrene-6,12-quinone (see Chapter 3).

Toxicity of ROS



Examples of chemical-induced oxidative stress

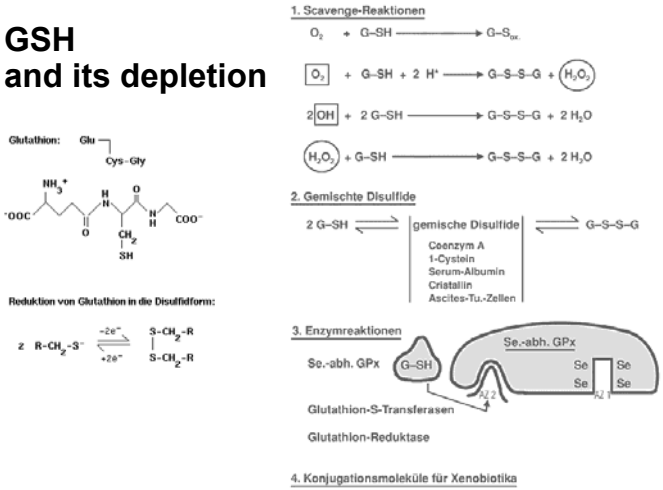
- Metals: fenton reaction -> OH*

- Depletion of GSH:

reactive molecules, GST-conjugation,
metals: SH oxidation ...

- Redox-cycling chemicals: oxy-PAHs

GSH and its depletion



Biomarkers of oxidative damage

Poškození	Produkt	Stanovení	Citace
fosfolipidy	**MDA**	TBARS assay, HPLC, HPLC s UV-detekcí	Draper et al. 1993, Bird et al. 1983, Selim 1977
DNA	**8-OH-dG**	HPLC, metoda s využitím imunoafinitní izolace	Degan et al. 1991, Loft et al. 1992
proteiny	**o-Tyr**	spektrofotometricky, HPLC, MS	Deneshvar et al. 1997, De Zwart et al. 1998

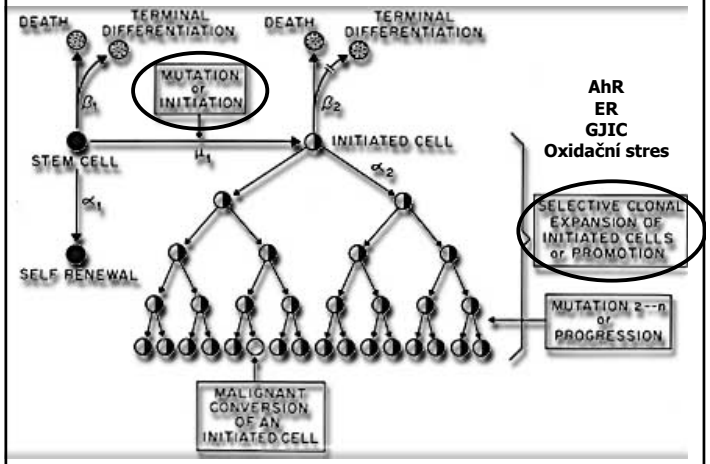
Vysvětlivky:
MDA malondyaldahyd
8-OH-dG 8-hydroxy-2[l]-deoxyguanozin
o-Tyr orthotyrozin
TBARS reaktivní látky s kyselinou thiobarbiturovou
HPLC vysokotlaková kapalinová chromatografie
MS hmotnostní spektrometrie

DNA damage mutagenicity and genotoxicity

DNA:
- **principal molecule for life of the cell**
- **structure and function carefully checked**
- **changes rapidly repaired**
- **irreversible changes -> cell death** *(apoptosis)*

Mutagenesis
- changes in the sequences of deoxynucleotides
  - deletions/insertions: changes in reading frame
  - exchanges of nucleotides: changes in aminoacids
- natural mutations (billions of nucleotides/day)
  : variability in genoms; reparations
- **chemical-induced mutagenesis**

IMPORTANT PROCESSES IN CANCEROGENESIS



Trosko and Ruch 1998, Frontiers in Bioscience 3:d208

Chemical induced DNA damage

Bases analogs

- incorporation into DNA during replication (5-Br-Uracil: AT -> GC)

HNO_2, HSO_3^-, Hydroxylamine, Methoxyamine-

- deamination of bases (GC -> AT)

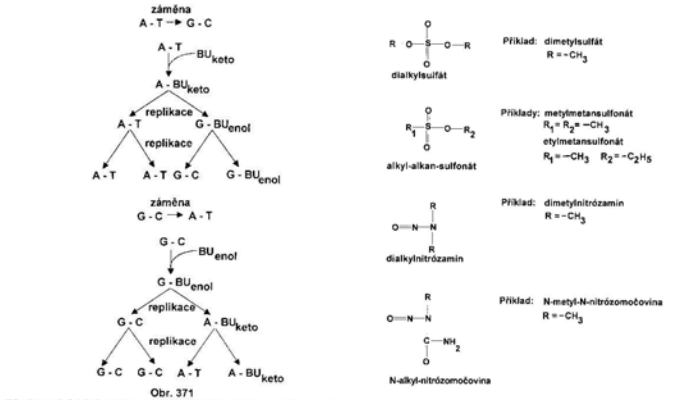
Alkylsulphates, N-nitroso-alkyles, cis-platinum

- alkylation of bases; crosslinks of dsDNA

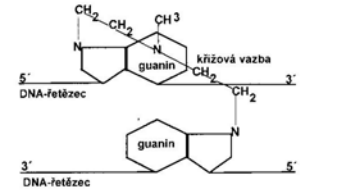
Polycyclic aromatic hydrocarbons (PAHs) & derivatives
(N-acetyl-2-aminofluorene (AAF), benzo[a]pyrene)

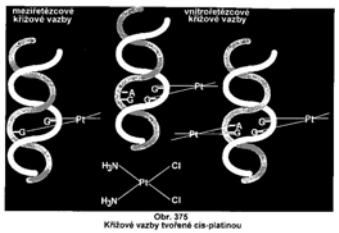
Mycotoxins (aflatoxins)

- require metabolic activation by CYPs
- aduct formation with DNA (*biomarkers*)

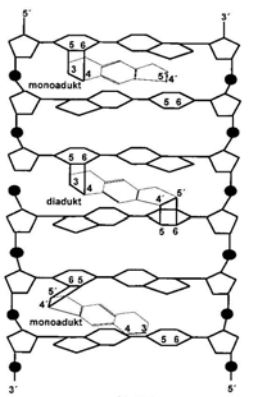


Obr. 371
Záměny párů bází v DNA pod mutagenním účinkem brómuracilu





Obr. 376
Křížové vazby tvořené cis-platinou



Obr. 377
Interakce psoralenu s DNA za tvorby monoaduktů a diaduktů

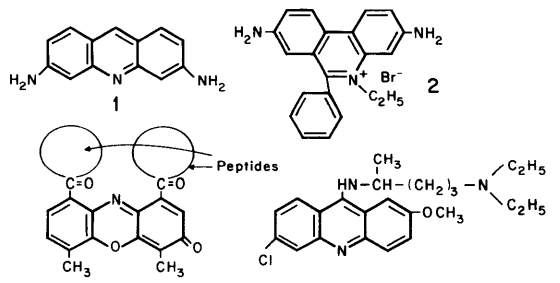
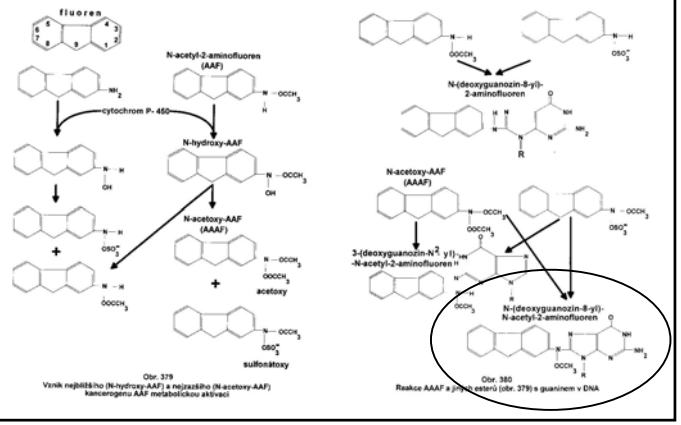


Chart 5.8. Examples of intercalating agents. Key: 1, acriflavine; 2, ethidium bromide; 3, actinomycin; 4, quinacrine.

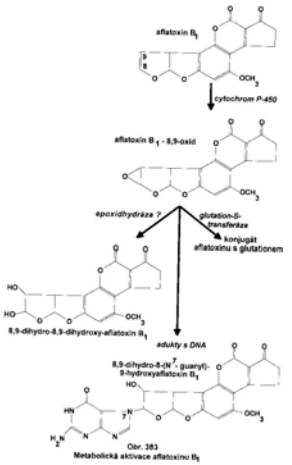
Metabolic activation of PAH and DNA-aduct formation



Obr. 379
Vznik nejbližšího (N-hydroxy-AAF) a nejzazšího (N-acetoxy-AAF) kancerogenu AAF metabolickou aktivací

Obr. 380
Reakce AAAF a jiných esterů (obr. 379) s guaninem v DNA

Metabolic activation of aflatoxin and formation of DNA-aducts

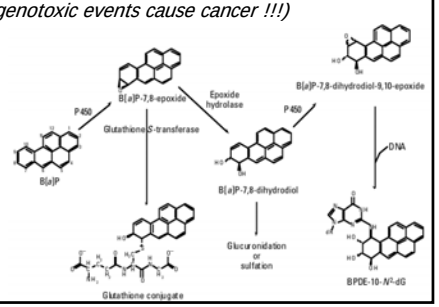


Obr. 383
Metabolická aktivace aflatoxinu B_1

Does **chemically-induced genotoxicity** has effects in vivo ?

- adducts from mitochondrial DNA ?
- distance between „source of radicals“ and nuclear DNA ?
- protection mechanisms (mutation -> death)

Rubin (2002) *Oncogene* 21:7392
Thilly (2003) *Nature Genetics* 34(3):255
Mutations are not caused by chemicals
Chemicals only allow „unveil“ previously existing mutations in nuclear DNA *(non-genotoxic events cause cancer !!!)*



Physical factors & DNA damage

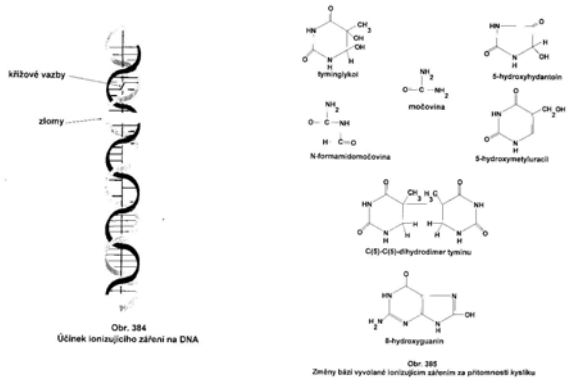
Ionizating radiation

- direct interaction with hydrogen atoms in water (and bases)
  -> OH* radicals; H_2O_2, O_2-
- oxidation of bases; dimerization ...

UV radiation

- interaction with aromatic cycles (bases)
- base dimerization (T=T)

Ionizing radiation effects on DNA



Obr. 384
Účinek ionizujícího záření na DNA

Obr. 385
Změny bází vyvolané ionizujícím zářením za přítomnosti kyslíku

DNA repair

Damage of DNA is carefully controlled

constitutively expressed proteins

Changes in DNA

induction of reparation enzymes ("SOS-repair")

= biomarker of DNA damage