

OXIDATIVE STRESS

INTRODUCTION

Oxidative stress is the result of an imbalance between oxidants (free radicals or reactive oxygen species (ROS) or reactive nitrogen species (RNS)) and defense systems (antioxidants), leading to structural and functional damage that is often irreversible for the cell. It is involved in most pathologies.

- **I/ Free Radical (FR):**

A free radical is a chemical species characterized by the presence of an unpaired (free) electron in its outer shell. This property makes free radicals capable of reacting with various molecules (they are oxidizing, aggressive, and highly reactive). Oxygen (O₂) and nitric oxide (NO) are highly available molecules at the cellular level; they undergo oxidation reactions, and a certain proportion is converted into free radicals (by partial reduction or sudden oxidation).

Free radicals are continuously produced in small amounts by various physiological mechanisms, as they are useful for the organism at reasonable doses. However, their production may become excessive, and the organism must protect itself through different antioxidant systems.

- **I/1. Nature of free radicals:**

Two types are distinguished:

A/ Reactive Oxygen Species (ROS): Radical oxygen derivatives (OH•, O₂•⁻, ...) + strongly oxidizing non-radical compounds (O₃) and/or easily transformed into radicals (H₂O₂), as well as free radicals derived from macromolecule oxidation: (RO•, ROO•).

B/ Reactive Nitrogen Species (RNS): Radical derivatives (NO•) or non-radical derivatives (NOO⁻).

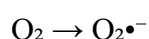
- **I/2. Formation of free radicals:**

A/ Reactive Oxygen Species (ROS):

→ **Radical oxygen derivatives:**

1- Superoxide anion (O₂•⁻):

Mainly formed during mitochondrial respiration and oxidative metabolism (P450) by partial reduction of oxygen (which receives 2 e⁻ instead of 4 e⁻).



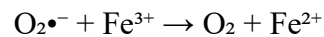
Superoxide anions are reactive and can diffuse from their subcellular production site to other cellular compartments (even the whole cell). They can react with various enzymes, unsaturated fatty acids (FA), and nucleic acids.

2- Hydroxyl radical (OH•):

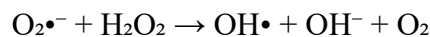
It is the most reactive oxygen free radical, which explains its high toxicity. It has very low diffusion and half-life but interacts instantly with sugars, amino acids, lipids, phospholipids, and nucleic acids.

It can be generated by several mechanisms:

a) Fenton reaction: decomposition of H₂O₂ in the presence of metals (M^{+x}) such as Fe²⁺



b) Haber-Weiss reaction: interaction of H₂O₂ with superoxide anion O₂•⁻



c) Homolytic cleavage of H₂O₂ under UV radiation

d) Reaction of hypochlorous acid with superoxide anion O₂•⁻

e) Decomposition of peroxynitrite ions (ONOO⁻)

The hydroxyl radical (OH•) can oxidize a substrate according to 3 different mechanisms.

3- Alkoxyl radical (RO•), Peroxyl radical (ROO•):

Highly reactive; they are formed during the attack of macromolecules by free radicals and during xenobiotic metabolism (CCl₄, paracetamol).

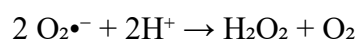
→ **Non-radical oxygen derivatives:**

1- Singlet oxygen (¹O₂):

Mainly formed during physicochemical processes (UVA). It represents the excited state of molecular oxygen (O₂) due to inversion of peripheral electron spins (resulting in antiparallel spins). It is highly unstable and extremely reactive. It produces superoxide O₂•⁻.

2- Hydrogen peroxide (H₂O₂):

A molecular (non-radical) species with redox properties. Its main production results from the dismutation of O₂•⁻ by superoxide dismutase (SOD) according to the following reaction:



It interacts directly with macromolecules or indirectly (it is a precursor of free radicals, producing $\text{OH}\cdot$ via the Fenton reaction). It is weakly reactive in the absence of transition metals (Fe^{2+} , Cu^+).

It diffuses rapidly through cellular membranes and can be eliminated into O_2 and H_2O by catalase.

3- Ozone:

Its formation requires a high energy input to the oxygen molecule (O_2), provided by ultraviolet radiation or electrical sparks (endothermic reaction).

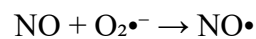
It is a powerful oxidizing agent of lipid chains, a generator of hydrogen peroxide (H_2O_2), and a precursor of aldehydes.

B/ Reactive Nitrogen Species:

Nitric oxide is found at high concentrations, especially in the brain, where it is synthesized by mitochondrial NO synthase for mediation purposes.

It plays a role in blood coagulation, vasodilation, neurotransmission, neuronal plasticity, intra- and intercellular signaling, defense mechanisms, apoptosis, and smooth muscle relaxation.

1- Nitric oxide radical ($\text{NO}\cdot$):



2- Peroxynitrite (ONOO^-): Has oxidizing activity and generates $\text{OH}\cdot$

3- Nitroperoxide (ONOOH)

- **I.3. Sources of free radical production:**

a) Endogenous sources:

1- Enzymatic:

- **Mitochondrial respiratory chain:**
Reduction of molecular oxygen by cellular respiratory cytochromes is accompanied by parallel production of superoxide anion $\text{O}_2\cdot^-$ (~2%), hydrogen peroxide H_2O_2 , and hydroxyl radicals $\text{OH}\cdot$.

Production increases during intense respiration (physical exercise) or mitochondrial disorders (genetic, inflammatory, nutritional such as ubiquinone deficiency).

- **Microsomal NADPH cytochrome P450 system (ER):**
+++ CYP2E1: auto-oxidation of the O₂-cytochrome P450 (Fe²⁺) complex releases superoxide O₂^{•-}

Some substances stimulate oxygen reduction by cytochrome P450 (e.g., paracetamol → NAPQI).

- **Inflammation:**
NAD(P)H oxidase: reduces O₂ to O₂^{•-} during phagocytosis
NO synthase (I, II, III, n): produces NO[•]
Myeloperoxidase (MPO): converts H₂O₂ into hypochlorous acid (HClO)
- **Other enzymes:**
Xanthine oxidase: ATP catabolism → O₂^{•-}
Monoamine oxidase: → H₂O₂
Arachidonic acid metabolism enzymes: COX

2- Non-enzymatic:

- Transition metals: Fenton and Haber-Weiss reactions
- Ischemia/reperfusion shock
- Auto-oxidation of adrenaline, dopamine, flavins, hydroquinone, hemoglobin → O₂^{•-}, H₂O₂, OH[•]

b) Exogenous sources:

1- Chemical agents:

- Toxic metals: Cu, Fe → OH[•] via Fenton reaction

$$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^{\bullet} + \text{OH}^- + \text{Fe}^{3+}$$

$$\text{H}_2\text{O}_2 + \text{Cu}^+ \rightarrow \text{OH}^{\bullet} + \text{OH}^- + \text{Cu}^{2+}$$
- Arsenic: H₂O₂ production during oxidation As³⁺ → As⁵⁺
- Lead, cadmium
- Inhaled particles: asbestos, silica
- Ethanol, tobacco, drugs
- Drugs: paracetamol, barbiturates
- Pesticides: paraquat
- Solvents: carbon tetrachloride

2- Physical agents:

- Ionizing radiation: water radiolysis → OH• (65% of effects)
- UV radiation: formation of singlet oxygen ($^1\text{O}_2$)

3- Microbiological agents

- **I.3. Actions of ROS:**

a) Physiological role:

- Second messengers and regulators of physiological processes
- Signal transduction, gene regulation, metabolic modulation
- Embryonic development, proliferation, differentiation
- Antibacterial defense
- Tumor cell apoptosis

b) Toxic actions: oxidation of macromolecules

1- Protein oxidative damage:

Targets: sulfur-containing and aromatic amino acids

Effects:

- Structural changes (cross-linking, fragmentation)
- Enzyme dysfunction
- Loss of specificity
- Formation of AGE
- Inflammation
- Protein accumulation

2- Lipid oxidative damage: chain peroxidation

Products: ROO•, ROOH, aldehydes (4-HNE, MDA)

Consequences:

- Loss of membrane fluidity
- Membrane dysfunction
- Cytotoxicity, mutagenicity, atherogenesis

3- DNA oxidative damage:

Consequences:

- Mutations
- Strand breaks
- Apoptosis if repair fails

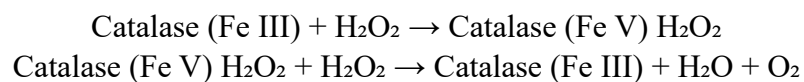
- **II. Antioxidants:**

Definition: Substances capable of inhibiting oxidation at low concentration by donating electrons.

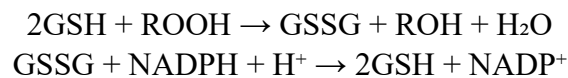
II.2. Antioxidant systems:

a) Enzymatic system:

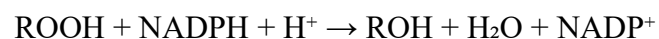
- **SOD:** removes $O_2^{\bullet -}$
- **Catalase:**



- **GPx / GR system:**

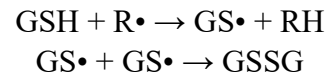


- **Thioredoxin system:**



b) Non-enzymatic system:

- **Glutathione (GSH):**



- **Vitamins:** E, C, A (β -carotene)
- **Trace elements:** Selenium, Zinc
- **Transport proteins:** Transferrin, ferritin, albumin

- **III. Evaluation of oxidative stress:**

- Samples: blood, urine
- Prooxidant markers: MDA, 4-HNE, 8OHG
- Antioxidant markers: vitamins, GSH, SOD
- Total antioxidant capacity: ORAC
- Iron status: serum iron, ferritin