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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 (O_2) 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 ($OH^{\bullet}$, $O_2^{\bullet -}$, …) + strongly oxidizing non-radical compounds (O_3) and/or easily transformed into radicals (H_2O_2), as well as free radicals derived from macromolecule oxidation: ($RO^{\bullet}$, $ROO^{\bullet}$).

B/ Reactive Nitrogen Species (RNS): Radical derivatives ($NO^{\bullet}$) or non-radical derivatives (NOO^-).

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

A/ Reactive Oxygen Species (ROS):

→ Radical oxygen derivatives:

1- Superoxide anion ($O_2^{\bullet -}$):

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

$$O_2 \rightarrow O_2^{\bullet -}$$

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 H_2O_2 in the presence of metals (M^{+x}) such as Fe^{2+}

$$H_2O_2 + Fe^{2+} \rightarrow OH\bullet + OH^- + Fe^{3+}$$

$$O_2\bullet^- + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

b) Haber-Weiss reaction: interaction of H_2O_2 with superoxide anion $O_2\bullet^-$

$$O_2\bullet^- + H_2O_2 \rightarrow OH\bullet + OH^- + O_2$$

c) Homolytic cleavage of H_2O_2 under UV radiation

d) Reaction of hypochlorous acid with superoxide anion $O_2\bullet^-$

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 (CCl_4, paracetamol).

→ Non-radical oxygen derivatives:

1- Singlet oxygen ($^1O_2$):

Mainly formed during physicochemical processes (UVA). It represents the excited state of molecular oxygen (O_2) due to inversion of peripheral electron spins (resulting in antiparallel spins). It is highly unstable and extremely reactive. It produces superoxide $O_2\bullet^-$.

2- Hydrogen peroxide (H_2O_2):

A molecular (non-radical) species with redox properties. Its main production results from the dismutation of $O_2\bullet^-$ by superoxide dismutase (SOD) according to the following reaction:

$$2\ O_2\bullet^- + 2H^+ \rightarrow H_2O_2 + O_2$$

It interacts directly with macromolecules or indirectly (it is a precursor of free radicals, producing OH• 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 (NO•):

$$NO + O_2^{\bullet -} \rightarrow NO^{\bullet}$$

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^{-} \text{ (peroxynitrite ion)}$$

2- Peroxynitrite ($ONOO^-$): Has oxidizing activity and generates OH•

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 $O_2^{\bullet -}$ (~2%), hydrogen peroxide H_2O_2, and hydroxyl radicals OH•.

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 O_2–cytochrome P450 (Fe^{2+}) complex releases superoxide $O_2^{\bullet -}$

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

- **Inflammation:**
  NAD(P)H oxidase: reduces O_2 to $O_2^{\bullet -}$ during phagocytosis
  NO synthase (I, II, III, n): produces NO•
  Myeloperoxidase (MPO): converts H_2O_2 into hypochlorous acid (HClO)

- **Other enzymes:**
  Xanthine oxidase: ATP catabolism → $O_2^{\bullet -}$
  Monoamine oxidase: → H_2O_2
  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 → $O_2^{\bullet -}$, H_2O_2, OH•

b) Exogenous sources:

1- Chemical agents:

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

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^- + Fe^{3+}$$
$$H_2O_2 + Cu^{+} \rightarrow OH^{\bullet} + OH^- + Cu^{2+}$$

- Arsenic: H_2O_2 production during oxidation $As^{3+} \rightarrow As^{5+}$
- 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 ($^1O_2$)

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:**

$$\text{Catalase (Fe III)} + H_2O_2 \rightarrow \text{Catalase (Fe V) } H_2O_2$$
$$\text{Catalase (Fe V) } H_2O_2 + H_2O_2 \rightarrow \text{Catalase (Fe III)} + H_2O + O_2$$

- **GPx / GR system:**

$$2GSH + ROOH \rightarrow GSSG + ROH + H_2O$$
$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

- **Thioredoxin system:**

$$ROOH + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

b) Non-enzymatic system:

- **Glutathione (GSH):**

$$GSH + R\bullet \rightarrow GS\bullet + RH$$
$$GS\bullet + GS\bullet \rightarrow GSSG$$

- **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