Cell Biology and Function (CBF)

Lecture 22

Oxygen toxicity

Free Radical injury & Anitoxidants

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Objectives:

- Enumerate ROS, RNOS & Describe generation of ROS.
- Describe ROS effect on body biomolecules.
- List diseases associated with ROS.
- Describe the body defence mechanism against ROS.

Definition:

It is any atom having one or more unpaired electron in the outermost shell (the unpaired electrons are highly energetic). Therefore, the free radical will be very reactive and unstable.

Mechanism of action:

Free radicals are highly reactive due to presence of unpaired electron. A single unpaired (excited) electron in the outer orbit is searching to become part of paired set and will "steal" an electron from another nearby atom to accomplish this pairing.

During this, the original free radical become stable but the neighboring atom, by losing an electron, becomes a free radical itself.

The new free radical will then seek out another atom to steal from and so on.

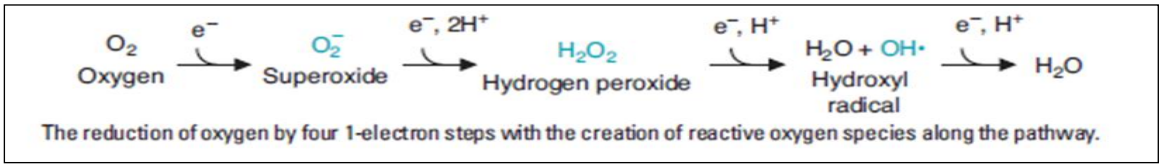
Types of Free radicals:

A) Oxygen – derived free radicals [Reactive Oxygen species (ROS)]:

1- Super oxide anion free radical ($O_2^{\bullet -}$)

2- Hydrogen peroxide (H_2O_2) not a free radical but can generate them.

3- Hydroxyl radical (OH•)



The reduction of oxygen by four 1-electron steps with the creation of reactive oxygen species along the pathway.

B) Nitrogen – derived free radicals [Reactive Nitrogen - Oxygen species (RNOS)]:

1- Nitric oxide ($NO^{\bullet}$)

2- Nitrogen dioxide ($NO_2^{\bullet}$)

C) Lipid – derived free radicals:

They include lipid free radicals **($L^{\bullet}$)**, lipid peroxide **($LOO^{\bullet}$)**, lipid hyperoxide **(LOOH)** & Malondialdehyde **(MDA)**

Although oxygen free radicals are the most reactive free radicals, their danger is limited because they are short –living, oppositely, Lipid free radicals are more dangerous because they are weaker & long living so, they attack more important molecules particularly DNA.

D) Xenobiotic – derived free radicals:

Several toxic, mutagenic, carcinogenic chemical compounds that enter the human body from the environment (exogenous source) are already in a free radical from or are converted by xenobiotic metabolizing enzymes into reactive species.

Sources of Free radicals:

I- Endogenous sources:

1. **Mitochondrial ETC:** CoQ is the major source of superoxide within the cell.
2. **Cytochrome P_{450}- mono-oxygneases:** This group of enzymes is involved in the detoxification of various drugs that enter the body. Consumption of alcohol and certain drugs induces expression of theses enzymes. So, patients who abuse such substances are more prone to the deleterious effects of ROS formed by these enzymes.

3. **Oxidases and oxygenases enzymes** e.g. **xanthine oxidase**& **peroxisomal oxidation of fatty acids** produces H_2O_2.

4. **Activation of cells of immune system** during phagocytosis of invading microorganisms, they utilize NADPH oxidase, myeloperoxidase enzyme & nitric oxide synthase to generate massive amounts of **superoxide $O_2^{\bullet -}$, hypochlorus acid (HOCl $^{\bullet}$) & Nitric oxide (NO $^{\bullet}$),** respectively. These kill phagocytosed microorganisms.

Clinical Correlates:

Chronic granulomatous disease (CGD) results from a deficiency of NADPH oxidase and the inability to effectively kill engulfed microbes, especially bacteria. Patients with CGD present with serious recurrent bacterial infections.

5. **Auto-oxidation reactions:** Transitional metals, Fe^{+3} & Cu^{+2} help formation of hydroxyl radical.

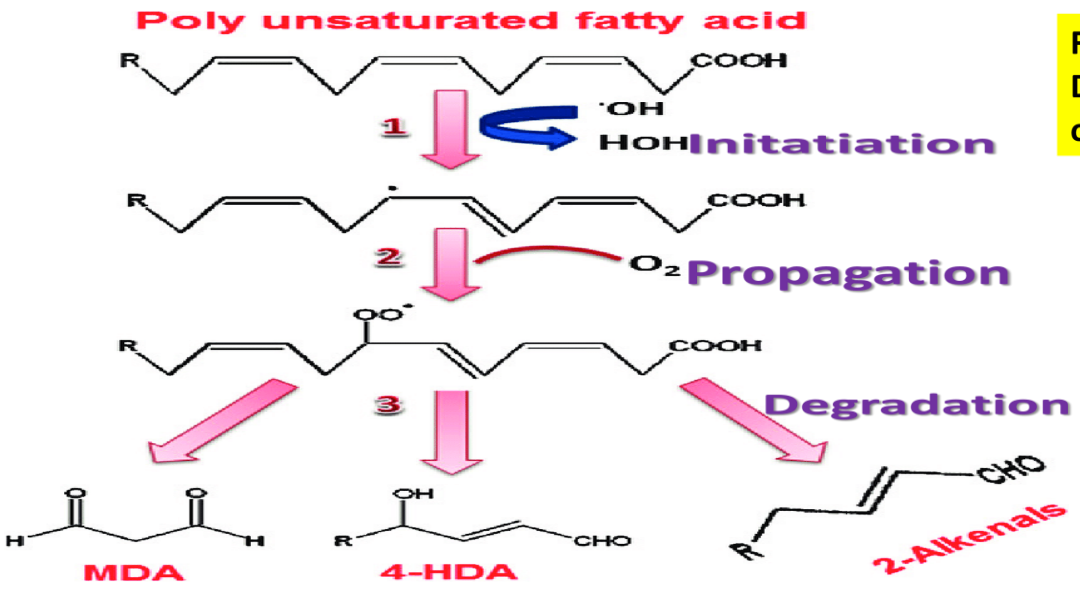
II- Exogenous sources:

1. **Cigarette smoke** → all types of free radicals.
2. **Ozone (O_3)** → major pollutant of heavily polluted cities.
3. **Ionizing radiation** → X and γ-rays → $HO^{\bullet}$.
4. **Non-ionizing radiation** → UV rays.
5. **Toxins**, e.g. benzopyrene and aniline dye.
6. **Air pollution** by incomplete ignition of fuel.

Damaging Effects of Free radicals:

A. Damage to lipid (lipid peroxidation):

- This **peroxidation** affects **polyunsaturated fatty acids (PUFAS)**. So, it affects unsaturated phospholipids, glycolipids and cholesterol in **cell membranes**.
- This is followed by **propagation (Chain reaction)** leading to formation of other lipid derived free radicals and **degradation** of these lipids into secondary oxidation products.
- **This ends in disturbance of membrane structure and functions** which results in increase membrane fluidity, change transport mechanisms and inactivation of membrane bound receptors and enzymes.



For Demonstration only

B. <u>Protein damage:</u>

This includes:

1. **Oxidation** of —SH groups in proteins

2. **Breakage** of the protein backbone

3. **Cross linkage** of proteins with each other and with other macromolecules.

This leads to:

a. Loss of biological activity of proteins i.e. enzymes.

b. Denaturation of structural protein → destruction of architecture of the cells and basement membrane → cell death.

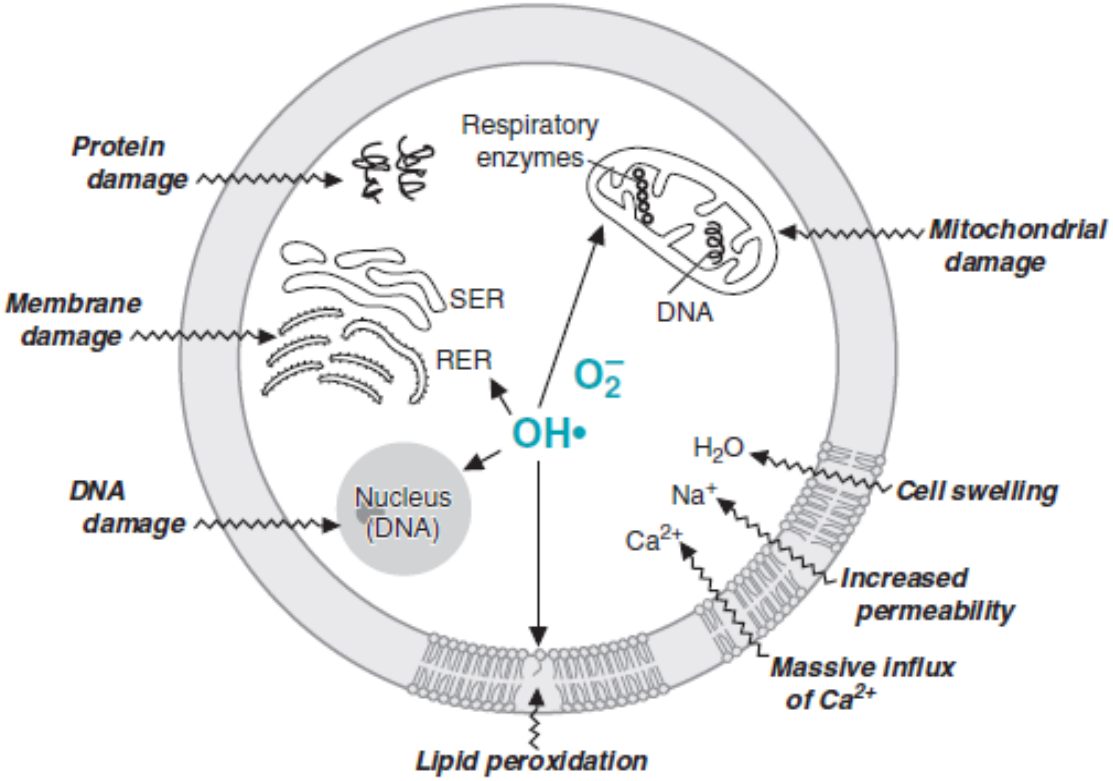
C. <u>DNA damage:</u>

This includes:

1. **Oxidation & Damage** of Bases & sugars.
2. **Breakage** of one or two strands of DNA.
3. **Cross linking** between DNA & DNA or DNA &protein.

This leads to:

Mutations followed by **cell death** or cellular transformation into **cancer.**



Diseases associated with Free radical injury:

In some cases, free radical damage is the direct cause of a disease state (e.g. tissue damage initiated by exposure to ionizing radiation), In other cases such as neurodegenerative diseases, (e.g. Parkinson's disease & in ischemia – reperfusion injury), ROS may perpetuate the cellular damage caused by another process.

1. Ischemic Reperfusion Injury.

2. Atherosclerosis.

3. Cancers: due to DNA mutations.

4. Aging: Neurodegenerative diseases, cancers and heart diseases

5. Prematurity dieases: Premature babies.

6. Immunological diseases: e.g. rheumatoid arthritis.

7. Diabetes Mellitus and its complications.

8. Eye injury: e.g. cataract and retinopathy.

9. Neurological diseases: e.g. Alzheimer's disease.

10. Renal failure.

11. Male infertility: decreased motility & abnormalities of spermatozoa.

12. GIT diseases: e.g. Inflammatory bowel diseases.

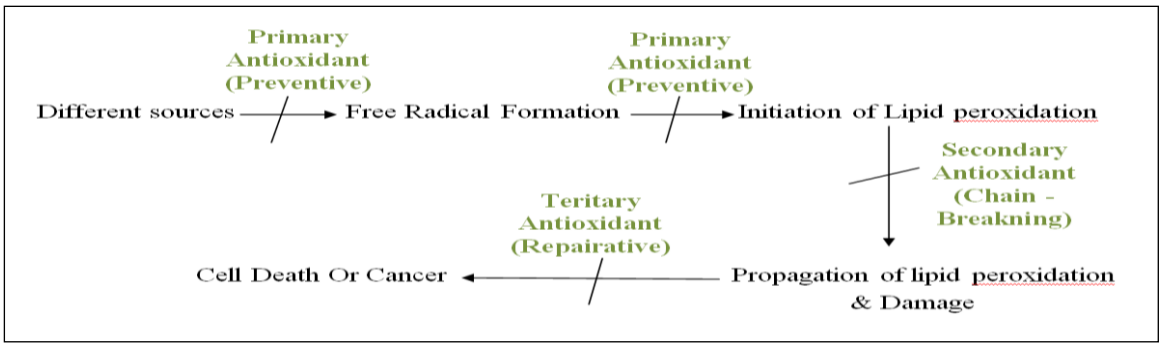
13. Hematological diseases: e.g. favism and anemia.

14. Respiratory diseases: e.g. emphysema.

Antioxidants

Definition:

It is any substance or mechanism that prevents generation of free radicals, scavenges free radicals or repairs damage induced by free radicals.



Classification:

1- <u>Primary antioxidants</u>: (preventive)

Role: They prevent generation of free radical or convert existing free radicals into less harmful molecules before they have chance to induce lipid peroxidation.

A. Chelating proteins for transition metals: They prevent the existence of free iron or copper and thus prevents the iron or copper catalyzed free radical formation. e.g. ferritin & tranferrin for iron and ceruloplasmin for copper.

B. Enzyme antioxidants:

They include:

1- Superoxide Dismutase (SOD):

Cu^{+2}, Zn^{+2} or Mn^{+2} dependant, it converts superoxide anion into H_2O_2.

2- Catalase:

Heme containing protein, It converts H_2O_2 into H_2O and O_2.

3- Glutathion peroxidase (GPX):

Two forms selenium dependent, selenium independent.

Use reduced Glutathione (GSH) as a coenzyme, it converts ROOH or H_2O_2 into ROH or H_2O.

2- <u>Secondary antioxidants</u>: (Chain - Breaking)

Role: They minimize lipid peroxidation by scavenging lipid free radicals preventing propagation. Secondary antioxidants give their own electrons to lipid free radicals (L˙), so, no longer need to attack the cell and the chain reaction is broken.

N.B.: Antioxidant has the ability to accommodate the change in electrons without become reactive.

-They include:

A. **Vitamins,** e.g. Vit. E, A, C & E which is the most potent

B. **Non – Vitamins:** include uric acid , bilirubin, glucose and proteins as albumin**.**

3- <u>Tertiary antioxidants</u>: (Reparative)

Role: They repair the damage occurred to target molecules or replace them with new ones.

- They Include:

1. DNA repair enzymes.
2. Methionine sulfoxide reductase.
3. Lysosomal enzymes responsible for hydrolyzing damaged molecules.

Oxidative Stress

Definition:

The generation of oxidant free radicals and the level of antioxidant defense systems are approximately balanced, the imbalance of oxidant free radicals/antioxidant systems is called oxidative stress. This imbalance is due either to:

1- Increase formation of reactive species. and/ or,

2- Decrease body-scavenging ability for these free radicals. and/ or

3- Increase the availability of transition metal ions.

Oxidative stress