Free Radicals and Antioxidants

By

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Objectives of the lecture:

- Definition of free radicals
- Types of free radicals
- Formation of reactive oxygen species
- External and internal causes of formation of free radicals
- Mechanism of damage.
- Antioxidant

The main references:

1-Lippincotts biochemistry (Richard A. Harvey).

2-Medical biochemistry (MN Chattergea,Rana Shinde)

3-biochemistry (Pankaja Naik)

Definition

A free radicals are atoms or molecules containing one or more unpaired electrons in their outer orbital. They are a highly reactive species and have high tendency either to loss an electron thereby acting as reducing agent or gain an electron acting as oxidizing agent thus they initiate chain reactions by extracting an electron from a neighboring molecules to complete their outer orbit. Free radical is generally represented by a superscript dot, (R•).

Formation of free radicals

The most important radicals are derived from molecular oxygen and certain oxides of nitrogen like nitric oxide. When the oxygen molecule is introduced into a reducing environment . It undergo a series of reactions leading to formation of reactive oxygen species , the reactive intermediate which are formed usually remain tightly bound in the active site of enzymes until the reaction is finished . They may escape from the active site of enzymes and lead to distractive effect

$$O_2 \xrightarrow{(+)e^-} O_2^- \xrightarrow{(+)e^-,2H^+} H_2O_2 \xrightarrow{(+)e^-,H^+} OH^\bullet \xrightarrow[(-)H_2O]{(+)e^-,H^+} H_2O$$

There two source from generation of free radicals

1- external source

2- internal source

External source include:

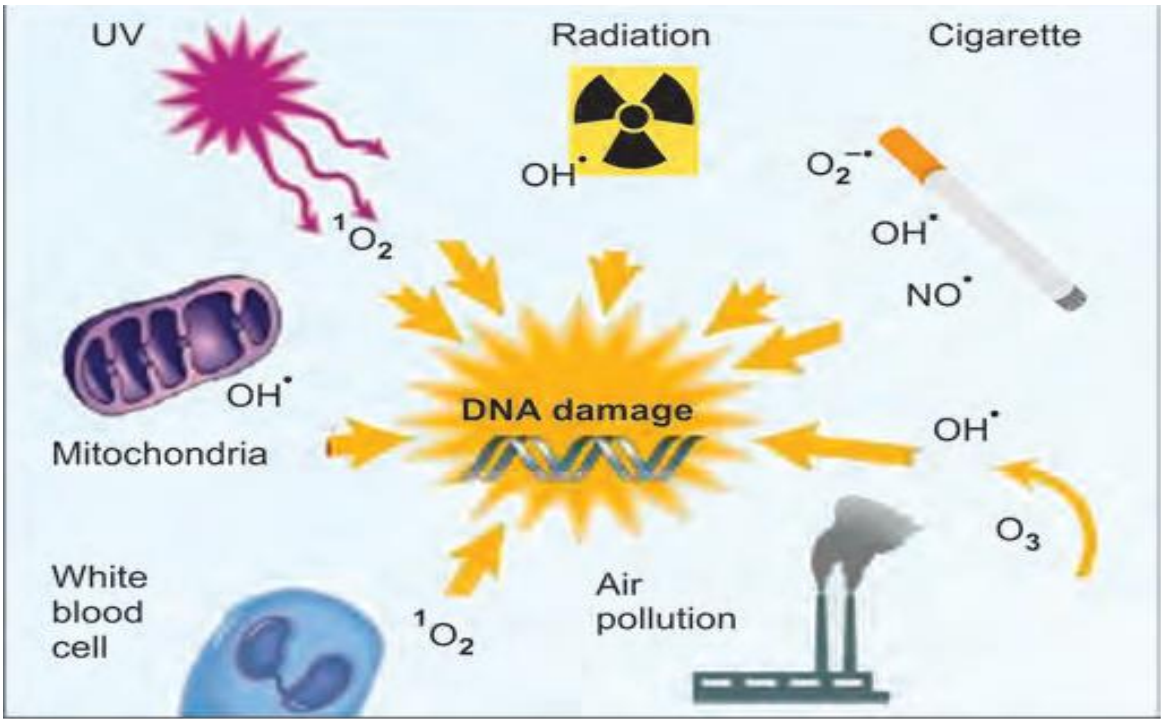
*lonizing radiation damages tissues by producing hydroxyl radicals, hydrogen peroxide and superoxide anion.

H_2O -------(gamma, UV radiation)----→ $H^\bullet + OH^\bullet$

*UV light and certain types of chemicals and drugs can produce FR.

*Cigarette smoke contains high concentrations of various free radicals.

*Inhalation of air pollutants will increase the production of free radicals



Internal sources

Enzymes reaction in which free radicals are by- products Various reactions occurring in the cells depend on a supply of oxygen in such type of reaction FR. are generated as by products these enzymes include:

1- Oxidases

2- mono-oxygenases

3- dioxygenases

Enzymes reaction in which FR are functional products. Functional FR may be involved in:

1- cellular signaling

2-cellular defense mechanism

Intracellular signaling FR

The FR nitric oxide (NO) is produced in various cells by a reaction catalyzed by nitric oxide synthase . NO produced act as an intracellular signaling molecule and performs many biological function include:

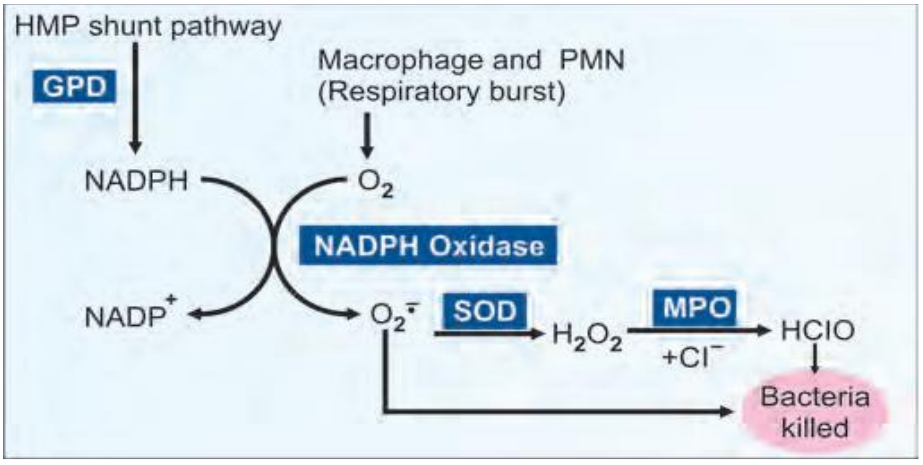
- Smooth muscle relaxation
- Killing bacteria and tumor cells
- Act as neurotransmitters

Has very short half life react with oxygen or superoxide and form proxynitrate ONOO which decomposes to form hydroxyl radical.

Cellular defense mechanism

Phagocytic cells from a large quantity of free radicals for killing bacteria. Phagocytic cells are activated and exhibit a rapid increase in oxygen consumption known as respiratory burst.

NADPH oxidase is located in the cell membrane of the inflammatory cells (neutrophils, eosinophils, monocytes and macrophages). Is activated upon contact with various ligand with receptor produces superoxide by a process of respiratory burst. The superoxide is converted to hydrogen peroxide and then to hypochlorous acid (HClO) with the help of superoxide dismutase (SOD) and myeloperoxidase (MPO). The superoxide and hypochlorous ions are the final effectors of bactericidal action



Clinical significant

Free radicals are responsible for the causation and progress of the following diseases :

- Atherosclerosis
- Some types of cancer
- Rheumatoid arthritis
- Ulcerative colitis
- Genetic deficiency of NADPH oxidase cause chronic granulomatosis (disease characterized by persistent and multiple infection of skin , lung , bone and liver)

Damage Produced by Reactive Oxygen Species

The main biological compounds that are damaged by the FR are

- Nucleic acids : lead to mutation
- Proteins lead to cellular malfunction due to structural changes
- Polyunsaturated fatty acids (PUF):

PUFA

- PUFA molecule reacts with free radical producing lipid free radical.

LH + OH• ------→ L• + H2O (Reaction 1)

(L•) (lipid free radical) rapidly reacts with molecular oxygen forming a peroxyl radical (LOO•)

- L• + O2 → LOO• (Reaction 2)

which can attack another polyunsaturated lipid molecule to produce lipid hydroperoxide and another lipiD free radical .

- LOO• + LH → LOOH + L• (Reaction 3)

This new lipid radical can , in turn be converted into anther lipid peroxy radical and lipid peroxidation processed as a chain reaction.

Antioxidants

Antioxidants are the compound that protect our body against toxic effects of free radicals .

Oxidative stress:

In the normal cells , there is an appropriate pro-oxidant and antioxidant balance . this balance can be shifted towards the pro-oxidants when production of ROS increase greatly (e.g following ingestion of certain chemical , drugs or exposure to ionizing radiation) or when the level of antioxidant diminished, this state is called oxidative stress.

Actions of antioxidant

1-prevent initiation of chain reaction by removing FR
2- scavenge FR generated by chain reaction thereby interrupting the chain sequence.
3- remove peroxide thereby preventing further generation of FR.

There are towlines of defense against FR:

1- enzymatic antioxidant system.

2- nonezymatic antioxidant system

Enzymatic antioxidant system:

1- Superoxide Dismutase (SOD).

2- Glutathione Peroxidase In the next step, the H2O2 is removed

by glutathione peroxidase. It is a selenium dependent enzyme.

The oxidized glutathione, in turn, is reduced by the glutathione reductase (GR), in presence of NADPH . This NADPH is generated with the help of glucose-6-phosphate dehydrogenase (G6PD) in HMP shunt pathway. Therefore, in GPD deficiency, the RBCs are liable to lysis, especially when oxidizing agents are administered (drug induced hemolytic anemia).

3- Catalase

- When H_2O_2 is generated in large quantities, the enzyme catalase is also used for its removal.

- $2\ H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2\ H_2O$

Non enzymatic antioxidant

1- vitamins (E,C and carotenoids)

Vit. E can break a chain process by reacting with lipid peroxide radical forming tocopheroxy radical (E•). tocopheroxy radical is stable because it is able to delocalized the unpaired electron with in its structure. Vit C and carotenoids are able to generate vit E from E•.

2- menerals (manganese, copper, zinc and selenium) these minerals are required for the activity of superoxide dismutase, glutathione peroxidase

3- polyphenols.