~~SPEECH~~

FREE RADICALS AND ANTI-OXIDANTS

→ A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital

→ Represented by a superscript dot, ($R^{\cdot}$)

→ Products of partial reduction of oxygen – Reactive oxygen species (ROS)

Examples:

Superoxide anion radical (O_2^{-})
Hydroperoxyl radical ($HOO^{\cdot}$),
Hydrogen peroxide (H_2O_2),
Hydroxyl radical ($OH^{\cdot}$)
Lipid peroxide radical ($ROO^{\cdot}$),
Singlet oxygen ($^1O_2$),
Nitric oxide ($NO^{\cdot}$)
Peroxy nitrite ($ONOO^-$)

Characteristic features of ROS are

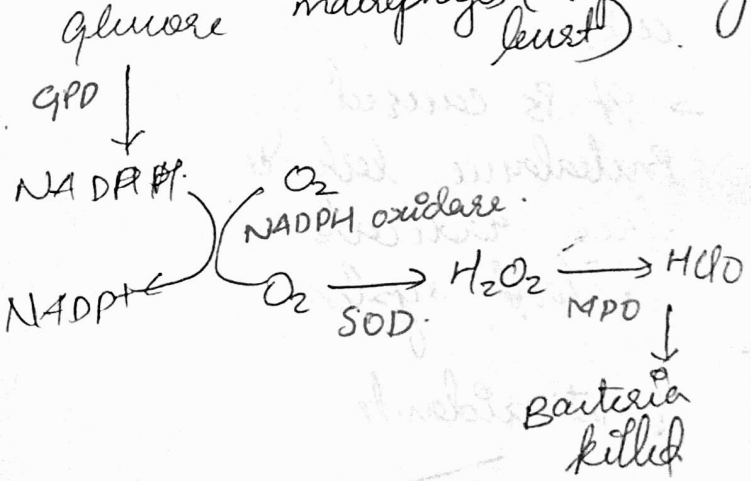
1. Highly reactive.
2. Very short half-life.
3. Generate new radicals by chain reaction
4. Damage to various tissues.

Generation of free radical.

→ Normal oxidation of food stuffs due to leaks in ETC in mitochondria.

→ Production of H_2O_2 and O_2 by oxidase enzymes (Xanthine oxidase and aldehyde oxidase)

→ During phagocytosis, inflammatory cells, particularly the macrophages produce superoxide ($O_2^{\cdot}$), by a reaction catalyzed by NADPH oxidase enzyme.



→ Macrophages also produce NO from arginine by enzyme nitric oxide synthase.

→ Peroxidation is catalyzed by lipo-oxygenase in platelets and leukocytes

→ Photolysis of oxygen by light

→ Ionising radiation damages tissues by producing hydroxyl radicals, H_2O_2 and O_2^-.

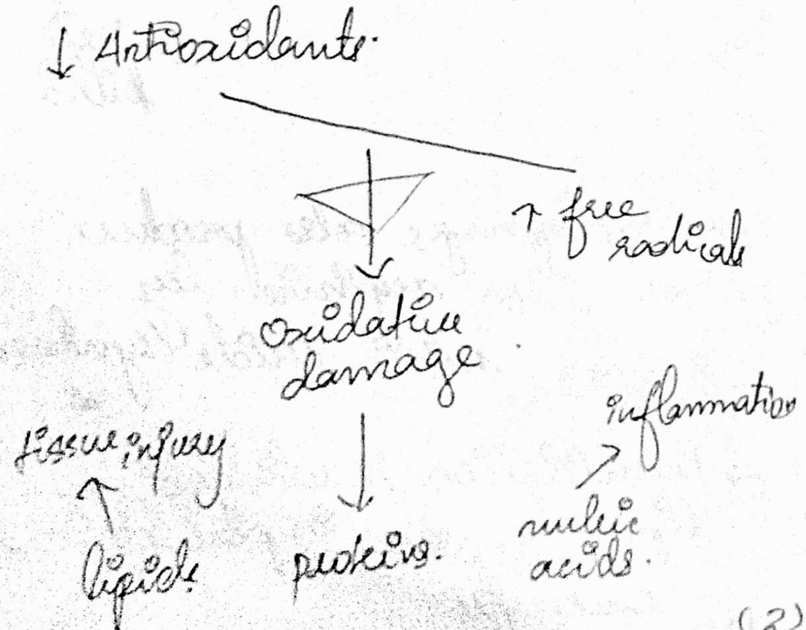
→ Cigarette smoke produce free radicals.

→ Alcohol, promoting lipid peroxidation.

Oxidative Stress. (4 mark)

→ Oxidative stress is a condition in which reactive oxygen species or free radicals (O_2^-, H_2O_2, OH^-) generated extra or intracellularly and exert toxic effects on cells.

→ It is caused by the imbalance between the free radicals and antioxidant systems of the body.



Lipid peroxidation.

① Initiation phase.

During this phase, interaction of PUFA with free radicals result in the production of R°

$$\underset{(PUFA)}{RH} + OH^\circ \longrightarrow R^\circ + H_2O$$

• The R° in turn is degraded to Malondialdehyde (MDA)

↳ Biochemical marker of oxidative stress

② Propagation phase.

→ The R° rapidly reacts with molecular oxygen forming a peroxyl radical (ROO°)

$$R^\circ + O_2 \longrightarrow ROO^\circ$$

ROO° then attack another Polyunsaturated lipid molecule

$$ROO^\circ + RH \longrightarrow \underset{(\text{Hydrogen peroxide})}{ROOH} + R^\circ$$

chain reaction (propagation) - one free radical generate another free radical in the neighbouring molecule.

(3) Termination phase.

The reaction continues till a peroxyl radical reads with another peroxyl radical to form inactive products.

$ROO^{\circ} + ROO^{\circ} \longrightarrow RO-OR + O_2$.

$R^{\circ} + R^{\circ} \longrightarrow R-R$.

→ The products of lipid peroxidation damage the membranes, cells and even tissues.

→ MDA - used as a biochemical marker for lipid peroxidation.

→ The estimation of serum MDA is often used to assess oxidative stress and free radical damage to the body

Harmful effects of free radicals:

→ Free radicals are capable of damaging biomolecules - Proteins, lipids, carbohydrates and Nucleic acids

Protein

ROS may cause fragmentation, cross-linking and aggregation of protein → loss of biological activity.

Lipids.

PUFA → lipid peroxidation

Carbohydrates.

Oxidation of monosaccharides → H_2O_2

Nucleic acid.

DNA mutation.

Free radicals and diseases

- Inflammatory diseases.
- Respiratory diseases.
- Diabetes
- cataract.
- Reperfusion injury.

ATP $\xrightarrow{\text{metabolism}}$ Hypoxanthine → Xanthine.

O_2 O_2°

H_2O ← ✗ H_2O_2.

- Peptic ulcer.
- Cancer
- Skin disease.
- Atherosclerosis.

(4 mark) Oxidative stress in Pathogenesis

Diabetes

→ Free radical formation

(3) In diabetes by non-

enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation
→ These leads to damage of enzymes, cellular machinery
→ Increased insulin resistance due to oxidative stress
→ Destruction of islets of cholesterol.

Atherosclerosis.

→ Free radicals damage blood vessel walls and cause atherosclerosis.
→ Low density lipoproteins (LDL) are deposited under the endothelial cells, which undergo oxidation by free radicals.
→ This attracts macrophages.
→ Macrophages are then converted into foam cells (absorbs and deposit LDL-cholesterol within the cell).
→ This initiates the atherosclerotic plaque formation.

Cancer

→ Free radicals produce DNA damage
→ Accumulated damages lead to somatic mutations and malignancy.

Antioxidants (Short note)

scavengers of free radicals.

→ Antioxidants are substances that protect cells from damage caused by free radicals.
→ They counteract oxidation and their role is to detoxify reactive oxygen species.
→ Antioxidants work by,
- Binding to the free radical
- Transforming them to harmless compound.
- Repairing cellular damage.

Mode of classification.

1. a) preventive antioxidants.
→ Block initial production of free radicals.
eg: catalase, Glutathione peroxidase, EDTA etc.

b) chain breaking antioxidant
→ Inhibit the propagative phase of lipid peroxidation
eg: SOD, vit. E, uric acid.

11. a) Endogenous Antioxidant (Enzymatic) (Short note)

1. Superoxide dismutase (SOD)
- Mitochondrial SOD – manganese dependant.
- cytoplasmic SOD. Copper - Zinc dependent
- convert superoxide ($O_2^{\cdot -}$) to H_2O_2 and O_2

2. Catalase.
- Marker enzyme for peroxisome.
- Remove H_2O_2 produced by SOD.

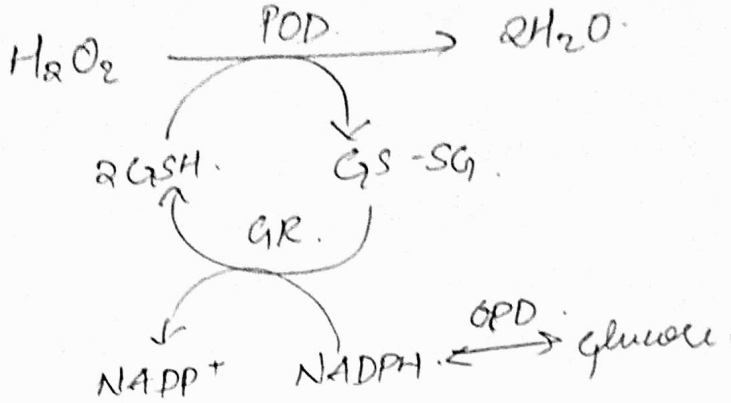
3. Glutathione peroxidase (POD)
- Detoxifies H_2O_2 to H_2O while reduced glutathione (G-SH) is converted to oxidized glutathione (GS-SG)
- The reduced glutathione can be regenerated by the enzyme glutathione reductase.

4. Glutathione reductase.

Antioxidant enzyme system

$$O_2^{\cdot -} + O_2^{\cdot} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

$$2\,H_2O_2 \xrightarrow{catalase} 2\,H_2O + O_2$$



b) Exogenous Antioxidants (Non-enzymatic)

1) Nutrient Antioxidants)

eg. α-tocopherol.
carotenoids (β carotene)
ascorbic acid & selenium.

a) Tocopherols (Vitamin E)
→ chain breaking antioxidant
→ interacts with peroxyl radical and inactivate it.
→ Protects from lipid peroxidation

$$T\text{-}OH + ROO^{\cdot} \longrightarrow TO + ROOH$$

b) Vitamin C (Ascorbic acid)
→ water soluble antioxidant in biological fluids.
→ Inhibit lipid peroxidation.

c) Carotenoids.

→ β carotene - chain breaking antioxidant

→ Lycopene & lutein: carotenoid pigments, act as antioxidants.

d) Selenium

→ Mineral present in the antioxidant enzyme Glutathione peroxidase.

→ Vit. E sparing action.

2) Metabolic antioxidants

~~Eg: Gluta~~

Eg: Glutathione, ceruloplasmin, albumin, bilirubin, transferrin, ferritin, uric acid.

<u>Function of antioxidants</u>

→ support kidney function

→ maintain good dental health

→ improve reproductive function

→ Improve nervous system functioning

→ have anti-aging effect

→ support immune system & improve defense power of the body.

→ protect liver.

→ reduce obesity

→ Offer protection against digestive disorders.

→ maintain healthy vision

→ Improve quality of sleep

→ support respiratory system