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MASTER OF MEDICINE PART- 1 COMMON CORE

Biochemical Aspects of Free Oxygen Radicals, Role in Diseases – An Overview

What is a Free Radical?

- Free radical is any atom or molecule that contains an unpaired electron in its outer electron orbital.
- Free radicals are electron-deficient species
- The unpaired electron makes the free radical unstable and extremely reactive
- Since electrons are most stable in pairs, free radical always try to stabilize itself by attacking other molecules or structures with high electron density.
- This creates another unstable free radical molecule that will also try to stabilize itself, thus starting a chain reaction that eventually damage cell structures and molecules.
- Free radicals are part of a group of Oxidants called Reactive Oxygen Species (ROS)
- Thus all Free Radicals are members of the group called Reactive Oxygen Species, but all Reactive Oxygen Species are not Free Radicals.

What are Reactive Oxygen Species (ROS)?

- Reactive Oxygen Species is a group made up of Free Radicals, Reactive Anions containing Oxygen atoms, or Molecules containing Oxygen atoms that can either produce Free Radicals or are chemically activated by Free Radicals.

List some Free Radicals and Reactive Oxygen Species in Human tissues.

- Some of the common free radicals and ROS in mammalian tissue include:]
- Superoxide radical ($\mathbf{O_2^{\cdot -}}$); Hydroxyl radical ($^{\cdot}\mathbf{OH}$); Per-hydroxyl radical ($\mathbf{HOO^{\cdot}}$) Peroxyl radical ($^{\cdot}\mathbf{ROO}$); Singlet Oxygen; Alkoxyl radical ($\mathbf{RO^{\cdot}}$); Hydrogen Peroxide ($\mathbf{H_2O_2}$); Nitric Oxide ($\mathbf{NO^{\cdot}}$), etc.

What are some of the sources of ROS (Pro-oxidants)?

- Chemicals and reactions that are capable of producing ROS in cells and tissues are called **Pro-Oxidants**.

- Sources of ROS (Pro-oxidants) can be separated into two major groups:
  - ROS that are naturally produced within human tissues
  - ROS that are produced externally (Environmental pollutants)

Production of ROS in human tissues (Pro-oxidants in human tissues):

- Some of the Pro-oxidants that are formed naturally in human tissues include: Superoxide radical, Hydrogen Peroxide and Hydroxyl radical. Examples of Pro-oxidants include the following:
  - Reactions in the Red Blood Cells.

- Example: Auto-oxidation of Hb to MetHb (about 3% of Hb in humans RBC is auto-oxidized per day) results in the formation of Superoxide radical

$$\{O_2 + e^- \text{======} \rightarrow O_2^{-\cdot}\}$$

  - Mitochondrial Respiratory Chain (Aerobic respiration)
  - Microsomal Cytochrome P 450 metabolism of Xenobiotics (Phases 1 & 2 reactions)
  - Cyclooxygenase and Lipoxygenase reactions
  - Auto-oxidation of Catecholamines and Xanthine Oxidase reactions
  - Beta-oxidation of fatty acids
  - Stimulation of Phagocytosis by Pathogens
  - Metabolism of Arginine, etc.

- **Some external source of ROS (Pro-oxidants):**
  - Environmental pollutants:-
  - Smoke and other components emitted by factories and motor vehicles.
  - Passive cigarette smoke,
  - Pesticides and Insecticides,
  - UV- radiation from the Sunrays.

How does the body disposes or neutralizes ROS (Pro-oxidants)?

- The body utilizes **Anti-oxidant** to dispose or neutralize ROS
- **Anti-Oxidants** are the compounds and reactions that are capable of:
  - Disposing of ROS,
  - Scavenging ROS,
  - Suppressing the formation of ROS or
  - Opposing the actions of ROS.

- Most antioxidants are electron donors, thus they react with Pro-oxidants to form products that are harmless to the tissues.

Give some examples of Anti-oxidants?

- Most of the know anti-oxidants can be put into one of three groups:
  - Enzymes that are present in tissues: Superoxide Dismutase (SOD); Glutathione Peroxidase; Catalase; Myeloperoxidase, etc.
  - Compounds present in tissues: Reduced Glutathione (GSH); Sulfhydryl (-SH) group, Alpha-Lipoic Acid; Ubiquinone (Co Q 10); Thioredoxin, etc.
  - Some essential nutrients: Vitamin C (Ascorbic acid); Vitamin E (d-alpha-tocopherol); Carotenoids; Bioflavonoids; N-acetyl Cysteine (NAC); Selenium (Se); Zinc (Zn) etc.

How does Anti-oxidants protect RBC from damage by Pro-oxidants (ROS)?

- In the RBC Oxygen can convert Hb to MetHb with the production of a Superoxide (Pro-oxidant)

$$Hb\text{-}Fe^{2+} + O_2 \text{ =======} \rightarrow MetHb\text{-}Fe^{3+} + O_2^-$$
(Superoxide)

- The enzyme Superoxide Dismutase (Anti-oxidant) in the RBC coverts the Superoxide (O_2^-) formed in the reaction to Hydrogen Peroxide (another Pro-oxidant) and Oxygen.

$$O_2^- + O_2^- + 2H^+ \text{ ========} \rightarrow H_2O_2 + O_2$$

- Reduced Glutathione (Anti-oxidant) in the RBC reacts with Hydrogen Peroxide (Pro-oxidant) to form H_2O and Oxidized Glutathione (GSSG). The enzyme Glutathione Peroxide that requires the trace element Selenium (Anti-oxidant) catalyzes this reaction.

$$2H_2O_2 + 2GSH \text{ ========} \rightarrow 2H_2O + O_2 + GSSG$$

- The GSSG is then reacts with NADPH to form GSH and $NADP^+$ in a reaction catalysed by Glutathione Reductase.

$$GSSG + NADPH + H^+ \text{ ======} \rightarrow 2GSH + NADP^+$$

- The Superoxide Dismutase (Anti-oxidant), Reduced Glutathione (Anti-oxidant) and Glutathione Peroxidase prevent the damaging effect that accumulation of Superoxide radical (Pro-oxidant) and Hydrogen Peroxide (Pro-oxidant) would have inflected on the RBC.

- GSH also protects the RBC from oxidation of –SH groups of the Haemoglobin molecules

What is the relationship between Pro-oxidants and Anti-oxidants in tissues?

- There is usually a balance between the **Pro-oxidants : Antioxidants** in tissues.
- A shift in this balance in favour of Pro-oxidants can occur when
  - Production of ROS (pro-oxidants) increases because of stress or the intake of certain drugs or chemicals with no corresponding increase in anti-oxidant intake.
  - Tissue levels of anti-oxidants are diminished, for example by:
  - Inactivation of SOD,
  - Reduced activity of Glutathione Peroxidase due to low intake of Selenium
  - Reduction of GSH or any other anti-oxidant such as: NADPH, Ascorbic acid, Vitamin E, Carotenoids, etc.

- The consequence of a shift in favour of Pro-oxidants is **Oxidative Stress**.

What is Oxidative Stress?

- By definition:
  - Oxidative Stress results when the rate of formation of Pro-oxidants is unbalanced in proportion to the protective Antioxidants in the tissues.
  - Oxidative stress is a general term used to describe a state of damage caused by ROS (Pro-oxidants).
- Damage can affect a specific molecule or the entire organism.

- Pro-oxidants, such as Free Radicals and Peroxides, represent a class of molecules that are derived from the metabolism of Oxygen and exist in all Aerobic organisms including Humans, thus they are regularly produced in the body.

What are the effects of ROS on Cells and Tissues?

- The main damage to cells and tissues results from the ROS-induced alteration of macromolecules and structures.
- Thus ROS is capable of:
    - Disruption of membrane integrity by reacting with Proteins and Polyunsaturated fatty acids in lipids in the Plasma membrane.
    - Causing alterations in Membrane Fluidity and Permeability and also changes in Activities of Receptors and Membrane-Bound Enzymes.
    - Causing damage to Proteins by Oxidation of -SH groups,
    - Stimulation of Phospholipases,
    - Inhibition of Na, K-ATPase, Adenylate Cyclase, Ca-ATPase,
    - Inhibition of other Channels and Pumps that are vital to cell metabolism
    - Damaging Nucleic acids by breaking DNA strands and modifying Nucleotide bases.

What are some of the diseases causes by ROS?

- ROS have been implicated in the development of at least 50 diseases.
- A partial list includes:
- Arthritis and other Inflammatory Diseases, Kidney disease, Cataracts, Inflammatory Bowel disease, Colitis, Lung Dysfunction; Pancreatitis; Drug reactions, Skin Lesions, and Aging, to mention a few.
- Heart disease and Cancer are two of the most widespread diseases associated with Free Radical Damage.

Role of ROS on Diseases:

- **Toxicity of Oxygen to Premature Infants**:-
    - Premature infants are often ventilated with High concentration of O_2 to compensate for their immature lung development.
    - Breathing high concentration of O_2 over a prolonged period of time can be dangerous because of increased production of SUPEROXIDE ($\mathbf{O_2^{-\cdot}}$).
    - Premature infants are particularly susceptible to damage because their capacity to produce Superoxide Dismutase (the enzyme needed to remove Superoxide) is not fully developed, therefore they are unable to produce sufficient enzyme to detoxify Superoxide.
    - The excess Superoxide in the cells of tissues can reacts non-specifically with and damage DNA and cell membranes.

- **G-6-P DH deficiency and Haemolytic Anaemia**:-
  - Glucose-6-Pphosphate Dehydrogenase (G-6-P D) is the enzyme that catalyzes the formation of NADPH in the Pentose Phosphate Pathway (HMP-shunt).
  - This pathway is very important because it is the major means of producing the NADPH that is required in the RBC and Hepatocytes.
  - A major function of NADPH in the RBC is to ensure the production of Reduced Glutathione (GSH) required for the removal of ROS formed during O_2 transport.
  - Individuals who are either deficient or have low levels of G-6-P D usually suffer from Haemolytic Anaemia.
  - The condition is more severe if these individuals consume certain drugs such as, the Anti-malarial drug PRIMAQUINE (Primaquine-sensitive haemolytic anaemia), Sulphonamides or Chemicals such as Naphthalene, or certain legumes (broad beans - Vicia faba) or some Food Additives...
  - The biochemical basis for the haemolytic anaemia is that the consumption of any of these materials leads to increased production of ROS (Pro-oxidants) in the cells.
  - RBC of individuals with low or deficient G-6-P D cannot generate sufficient NADPH to regenerate GSH from GSSG.
  - This affects their ability to dispose of ROS.
  - Accumulation of ROS in the RBC causes damage to the membrane lipids leading to Lysis of the cell membrane and haemolysis.

How important is Reduced Glutathione (GSH) in protecting cellular damage by ROS?

- GSH has been found to be low in many disease states (including virtually all those noted above) indicating Oxidative Stress and Inadequate Antioxidant activity.
- One suggestion is that maintaining and improving GSH levels may play significant role in combating some of these diseases.
- There are several ways to increase GSH levels:
  - GSH can be given as a Supplement but it is poorly absorbed in the GIT.
  - Cysteine is the main precursor for biosynthesis of GSH in tissues, but Cysteine is an unstable compound.
  - Cysteine can be obtained easily from the more stable compound N-Acetyl Cysteine (NAC), which is probably the best way to administer Cysteine.
  - N-Acetyl Cysteine is very effective in increasing GSH levels.
  - Alpha-Lipoic Acid and Vitamin C both increase internal recycling of GSH thus increase the GSH levels.

- GSH is important in the normal functioning of Immune Cells.
- Low GSH levels have been associated with Impaired Immune Function.
- Decreased GSH Impairs T-cell proliferation and activation.
- TNF-Alpha (a major pro-inflammatory Cytokine) impairs GSH production by several mechanisms, resulting in lowered GSH levels.
- Furthermore, oxidative stress increases TNF-Alpha production, thus GSH disturbances and enhanced TNF-Alpha production leads to a Pathogenic "Loop" or Vicious Cycle.