**ROS**

Last time we talked about pentose phosphate pathway which is an introduction to the Oxygen toxicity (ROS). You can either refer to the book or study the sheet and slides.

-Last time we talked about pentose phosphate pathway and we mentioned that one of its products is the NADPH which is the master source of electrons for either protective biosynthesis or detoxification of oxidants.

-simple definition of a radical is an unpaired electron which can be donated to others to produce a free radical OR can take up an electron from a neighboring molecule producing also a free radical.

**Referring to slide #1 :**

-last two electrons in the oxygen are not paired (biradical), this oxygen during its metabolisms and mobilization is a source to produce those ROS (superoxide ion, hydrogen peroxide[which is oxidizing substance and a source of free radicals but it is not a free radical]and hydroxyl free radical) along with them we also have reactive oxygen and nitrogen species.

-oxygen is essential for life however it also has toxicity that’s why we only give defined doses of oxygen.

**Slide #2:**

-oxygen that we breathe more than 90% goes to the respiratory chain for energy (ATP) production, and we have some other part that is utilized by enzymes (oxidase and oxygenase)

-oxidase usually gives off hydrogen peroxide and water.

-oxygenase we get mono or di where in mono it incorporates one oxygen atom as an electron acceptor molecule and di it incorporates both oxygen atoms.

-3-5% of oxygen goes to production of ROS.

-ROS are generated by normal metabolism but most are from environmental factors like infections(this is how microphages digest foreign organisms) radiation(x-rays and gamma) and chemicals and drugs, the process of aging, exposure to high oxygen tension, smog, cigarettes are full of ROS and free radicals => those will cause cell injury. If the ROS increases we’ll produce oxidative stress. We have other ROS like organic peroxide, hypochlorus acid (present in Clorox) and RNOS.

**#3:**

Those are some diseases that are associated with ROS (check the slide). ROS can affect most of our systems. So ROS can cause disease or they can contribute to complications to the chronic diseases.

-All metabolisms are affected by ROS but Amino Acids are the most affected since it would lead to fragmentation which would lead to aggregation of the protein and the aggregated protein is more likely to be digested.

-most susceptible amino acids: Pro, His , Arg, cys, Met

-Membrane lipids and DNA are also affected.

**Slide#6 :**

We know that oxygen when it is neutralized to water goes through ( O2🡪O2\*-🡪 H2O2🡪OH\* +H2O🡪 H2O) but this is protected by complex 4 nothing can escape no free radicals will escape.

-O2\*: superoxide radical

-OH\*: hydroxyl radical

The reason we don’t like excess iron Fe2+ is the Fenton reaction which is between iron (Fe2+ or Cu2+) and hydrogen peroxide which gives off hydroxyl radical and hydride ion.

We also have the superoxide ion and the hydrogen peroxide they can produce free radical by the Haber-Weiss reaction 🡪 OH\* from hydrogen peroxide.

**Slide#4:**

Okay, we said that those free radicals target variety of macromolecules, one of those targets is the membrane lipid which contain poly unsaturated fatty acids also they affect protein and DNA but we ll take PUFA as an example:

PUFA (LH) is attacked by hydroxyl free radical to produce lipid free radical (L\*) this lipid free radical will react with oxygen to produce peroxyl free radical and this peroxyl radical will attack another PUFA(LH) molecule and it produces another lipid free radical (chain reaction) to start the cycle again, but we also got lipid hydroperoxide as a product, this lipid hydroperoxide undergoes degradation ,those degraded products : 1- degraded lipid peroxide 2-malondialdehyde “MDA” (which is a bit harmful)

-this MDA is a very famous marker that is used to monitor oxidative stress in patients. There other markers but this is mainly used.

-One example is the diabetic patients which have higher MDA than normal people.

-now, when we have this damage, this damage affects all parts of the cell; membrane has lipid peroxidation which causes a channel or a gap which causes massive ion influx and water and cells burst,DNA undergoes damage, other intracellular membranes whether ER or mitochondria undergoes damage also the protein undergoes damage so the damage is widespread by these ROS.

**Slide#7:**

Sources of ROS:

A-We talked about oxidases; oxidases when they catalyze a reaction they can produce water or hydrogen peroxide. Mostly hydrogen peroxide. And we see these oxidases are in compartments that are equipped with protective antioxidant enzymes so that when the H2O2 are produced they are readily neutralized, because these H2O2 produced are the source of OH\* free radical by the fenton reaction we talked about earlier.

B-oxygenases;we talked about 1-monooxygenases which are (hydroxylases) and we have a big family of monooxygenases. 2- dioxygenases where two oxygens are incorporated,di oxygenases work in the production of those compounds : thromboxanes,PG (prostaglandins),Leukotrienes.

C-Coenzyme Q step in the respiratory chain produces oxygen species.

D-Respiratory burst

E- Ionizing radiation.

**Slide#8 :**

We showed this figure previously when we talked about monooxygenases,this is the cytochrome p450 enzyme which is a superfamily of many enzyme. The electrons are transferred from the NADPH to the FAD then FMN or directly to FMN from FE-S center ending to the acceptor and one oxygen goes to the acceptor where you form this hydroxyl free radical now in these reactions what happens unfortunately is that electrons can escape and bind with oxygen forming superoxide ions which will be converted to hydrogen peroxide or superoxide ion with hydrogen peroxide will for a hydroxyl free radical.

-in the R.C at this step of CoQ (refer to the slide ) there can be a accidental escape of electrons which can bind with oxygen in the media forming superoxide ion in the complex 4 this binuclear center prevents the release of free O2 radicals they do not escape.

**Slide #9 :**

Cytochrome P450 are present in the mitochondria and in the microsomal system, the slide shows the general reaction for them. This hydroxylation is necessary for solubilization of many drugs, chemicalcompounds, it is used for steroid synthesis vitamin d etc.

We have many reactions that involve hydroxylation in the mitochondria and in the microsomal system. And in all these reactions there is a possibility of release of different free radicals.

**Last slide:**

Infection is one big source of ROS,infection in the process if phagocytosis,Macrophages,Neutrophils and Eosinophils are involved in uptake of oxygen in a process we call respiratory burst , what happens is an enzyme called NADPH oxidase will be mobilized and this will bind to the membrane it utilizes oxygen to produce superoxide ion, this superoxide ion spontaneously or by superoxide dismutase enzyme is converted to H2O2,there some granules secreted that contains an enzyme (myeloperoxidase) which contains heme this enzyme binds the H2O2 with a chloride ion forming hypochlorus acid (this is the same material used in Clorox and other detergents),this HOCL in the presence of iron bye the Fenton reaction will produce free radicals. Also this superoxide ion will incorporate with another free radical called nitic oxide,to produce reactive oxygen nitrogen species, this nitric oxide is produced by nitric oxide synthase which is found in different places one of them is the immune cells and this enzyme is induced by infection,(peroxynitrite produces RNOS),now we ended up with RNOS,OH\*,HOCL and the NO itself all of these will attack the bacterial cell. (– you have to relate this to the G6PD deficiency)

**NO and RNOS**

-NO is a free radical

-it is an essential compound it has a function but it also is a free radical and diffuses readily. Check the sentences in the slide.

-The NO synthase synthesizes the NO in low amounts for its importance that was mentioned in the slide. We have different forms of this enzyme: isoenzyme1 ( neural) ,isoenzyme3 (endothelium),those enzymes do not undergo induction they have a constant amount to produce constant amounts of NO enough to produce its function with no danger.

But enzyme iNOS-isoform2 undergoes induction and produce large amounts of nitric oxide.

The next slide shows the synthesis of NO.

-pay attention to the cofactors: FMN,FAD,THB (tetrahydro byutirin)

The slide also shows what NO does.

