Biochem-lec 1

The oxidative stress and neurodegenerative diseases

This lec will discuss the definition of oxidative stress, association studies(how much the oxidative stress within blood, secretions out of the body and CSF) linking the oxidative stress with these diseases.

These diseases are more likely to occur in elderly, so aging is the most important factor in these diseases.

Stressed cells behave differently, so they start to age at a shorter time.

So, the total amount of oxidative stress in the stressed cells defines the age of the cells, defines the age of tissues and accordingly, aging of the body. Oxidative stress damages everything .even they start to correlate many diseases(like cardiovascular and neurodegenerative diseases and cancer) with oxidative stress.

Mechanism of action differs according to the disease and its pathology. But, generally oxidative stress start degrading molecules and cells, also fragmenting the mitochondria reducing the capacity of making energy. So, low energy capacity in aged people to perform their own processes.

Does the oxidative stress happen as a result of neurodegenerative diseases?, or does it cause these neurodegenerative diseases ? the answer of these questions will be discussed when studying the relation to neurogeneration. How does Oxidative stress affect the whole body? how does it specifically attack and cause certain diseases in old ages? and, if the oxidative stress is the cause and if it's fundamental to these diseases!! Can anti-oxidative treatment reverse the case? Or even stop the pathogenesis?

**This lec is based on the view article (mechanism of oxidative stress and neurodegeneration) " the reference"

Brain constitute 2% of the total body weight, consumes more than 20% of the whole body oxygen. So, high concentration of oxygen within the nervous system, as the oxygen is essential to all processes.

In oxygen reactions, when the product has free unpaired electron as free radical, or if the molecule has the potential to be converted to free radical. These collectively are called ROS.

In the past they considered ROS as a free radical. Nowadays, ROS are considered as any molecule which can be converted to free radical.

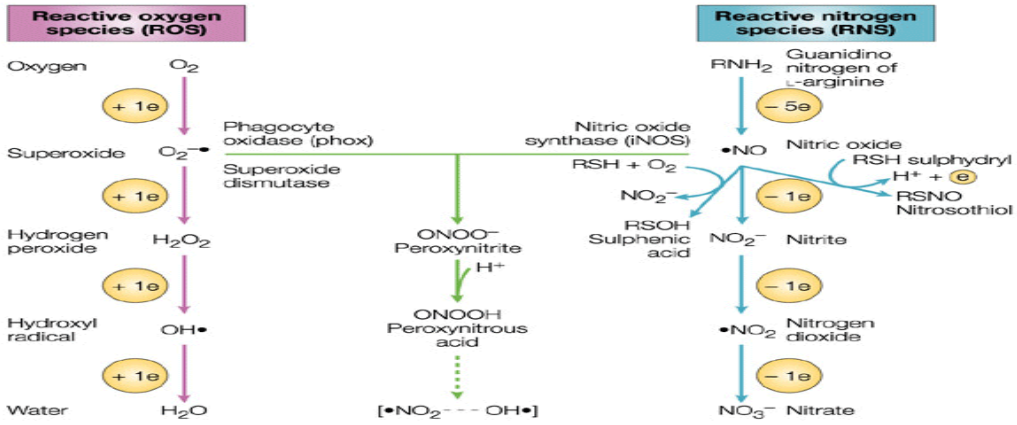
Groups of ROS: Superoxide molecule, hydrogen peroxide, hydroxyl radical which is the most reactive and most dangerous ROS, oxygen now is considered as ROS, ozone.

Anti-oxidant system or the oxidative stress issue within CNS is more important than other systems within the body, why? Because:1- the level of oxygen within CNS is much higher than others. This also explains why the oxidative stress affect CNS more than other systems.2- also the neurons are

non dividing cells so any defect that occurs in them will stay there for good.3. Nitric oxide and nitrogen is very high. So, fighting mechanism –anti-oxidant- within CNS should be more profound.

ROS attack lipids, proteins and nucleic acid. But, how do they attack? By trying to abstract electrons from everywhere. And This is the problem with free radicals –ROS- , they acquire electrons from anywhere. So, they attack very important proteins, lipids, DNA or RNA (they can disturb a very functional gene). They acquired the electron, to return the saturated number of electrons neglecting what has happened to the other molecule.

These are examples of ROS, and how do they convert to each other.



*the more oxidative stress you've, the more you should find lipids, proteins and nucleic acids which are broken randomly.

There are markers in lipids, proteins and nucleic acids which tell you that you've high level of oxidative stress. "Memorize the main markers as they're highly used in researches"

in lipids, the main markers are: MDA-malondialdehyde- and 4-hydroxy-2-nonenal . when ROS attack lipids you'll find high concentration of these two markers within the blood, brain and CSF.

In proteins, ROS start attacking the side chains of amino acids and backbone of protein randomly, ROS acquire the electron randomly, converting carboxylate group into aldehyde(it'll be converted into ketone group ,if it was between two R groups)- conversion into carbonyl group-. the more you've carbonyl group when testing the more is the oxidative stress.

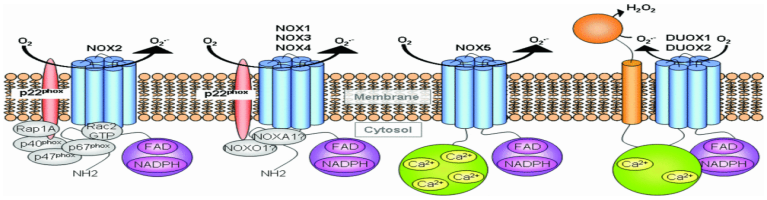
And any high level of hydroxylated purines or pyrimidines(cytosine),indicate high level of oxidative stress within the body. Hydroxy of any of the bases in DNA is a marker.

What are the molecules that produce ROS within the brain and the whole body?

1st. NADPH Oxidase:

Convert NADPH into $NADP^+$, during this process it produces superoxide(oxygen molecule has extra unpaired electron) . One group of this family called "NADPH oxidases" has 7 different genes coding 7

different proteins (NOX1,2,3,4,5 and DUOX1 and 2)



All what we've to know is: NADPH oxidase catalyze conversion from NADPH into $NADP^+$, coupled to this process is the production of superoxide.

2nd. Xanthine Oxidase:

This enzyme is responsible for uric acid production, so it's related to gout. Help in diagnosis of gout, where the level of this enzyme and uric acid is high.

This enzyme converts hypoxanthine into xanthine, and xanthine into uric acid- in two different subsequent rxns-. in these two rxns, there'll be conversion either from oxygen to superoxide, or oxygen to hydrogen peroxide, so in both the product is ROS. So, this enzyme is considered as another producer of ROS within the brain.

3rd.Mitochondria:

Mitochondrion is the most dangerous one in producing ROS, why? Because it contains high level of oxygen, from air>>blood>> cells(mitochondria; to final acceptor of electrons-complex 4-. so that, the affinity toward the oxygen is as follow: hemoglobin<myoglobin<complex4). Complex4 in electron transport chain has the highest affinity for oxygen, so it's the final acceptor of electrons; Collecting the oxygen within mitochondria. At the end, there'll be high concentration of oxygen and high concentration of electrons-the aim of Krebs cycle is to extract electrons, and to make sequence of electron movement- within mitochondria. So, the possibility to produce ROS is much higher. And it's active all the time. that's why it's considered as the most dangerous in producing oxidative stress.

**When will mitochondria produce ROS?

When any part of electron transport chain is blocked (complex4,3,2,1)>decreasing the movement of electrons, less oxygen is being used>> less oxygen is reduced to water-which means high level of oxygen in mitochondria-. Also increasing the electrons (more oxygen>>more electrons).> producing more ROS. Here, you might decrease the flow of electrons from Krebs cycle. But, it won't be effective enough, as there is still flow of electrons from cytoplasm through shuttles.

Production of superoxide depends on the membrane potential (difference between outside and inside) of mitochondria. What's the relation between superoxide production and membrane potential? Is increasing the production of superoxide related to high membrane potential? Or low membrane potential?

The process of electron transport chain: pumping of protons out (+ve charge outside) >>building up an electro-chemical gradient potential>>influx through ATPsynthase producing ATP. Because of ΔE the electrons move from one complex to another, so there's difference in energy, this difference in energy is used to pump protons out, this difference pump 4protons to outside in complex 1 and 3,

and 2protons in complex4. If the potential is high outside, so the difference in energy isn't enough to pump protons outside, causing stasis of electron movement. So that, there're inhibitors such as amino oligomycin; antibiotic inhibit ATPsynthase,cause inhibition of the electron influx through ATPsynthase ,stopping all electron transport chain because of building up of protons outside.

So, the more the potential, the less the movement of electrons, the higher the capacity of producing ROS. Decreasing the movement of electrons increase its concentration inside and decrease the oxygen that's used, so the possibility to produce ROS is higher.

Again, the mitochondrion is the most dangerous one, as 1-it's active all the time,2- it has the highest concentration of oxygen and the highest movement of electrons.

**Mitochondrial un-couplers help in decreasing the oxidative stress, how?

Their function is un-coupling, they cause the protons to pass through holes in the membrane from outside to inside instead of passing through ATPsynthase. They don't produce ATP. However, they produce heat and this is how we produce heat in our bodies –normally 37.

If we increase the concentration of un-couplers, we decrease the amount of oxidative stress within our bodies, so decreasing the possibility of having neuro-degenerative diseases.

4th Monoamine Oxidase:

Monoamine Oxidase A&B , 70% identical in structure .

They are oxidizing monoamines (epinephrine, dopamine and serotonin) ,they catalyze the oxidative deamination of monoamines within reaction in two subsequent steps (by each step they eliminate one electron to get rid of amine group) :

In step 1 they eliminate one electron using (FAD) to become FADH , in second step FADH become FADH2 , the end result is the production of aldehydes . after the aldehydes formation, FADH2 will donate the 2 electron to oxygen - to get back to the original FAD- producing H2O2 ,this is why monoamine oxidase are producers of oxidative stress in our bodies .

How do we fight oxidative stress? By antioxidant enzymes

Antioxidant enzymes:

1- Superoxide dismutase (SOD) have three isoforms:
   - Copper zinc SOD
   - Manganese SOD
   - Extracellular SOD (doesn't use any metal)

SOD convert superoxide molecule into hydrogen peroxide (H2O2)

2- Glutathione peroxidase

Converts (reduced glutathione) into (oxidized glutathione), this reaction is coupled with conversion of H2O2 into H2O

If the level of oxidative stress is high within the body , you will find low level of reduced glutathione.

3- Catalase converts H2O2 INTO water, it's the fastest enzyme ever, It can convert 10 million molecules of H2O2 into water per second, so the efficacy is high.
   The flow of oxygen happens all the time within your body, so you need a very effective antioxidant system in your body so as you can live.

Non-enzymatic antioxidants:

1- GSH the main antioxidant in CNS

The Glutathione structure is composed of three amino acids (glutamate, glycine and cysteine) characterized by a thiol group, so it can bind to another glutathione by disulfide bridge.

2- Vitamin E: lipid soluble molecule , it works as antioxidant .

It doesn't have specific mechanism to eliminate free radical. And it contains a ring structure.

The importance of ring: if you take an electron out from any of the periphery of the ring, you will saturate the free radical leaving the ring with free unpaired electron, resulting in resonance on the ring. So ring structure can stabilize free unpaired electron better than other structures, therefore any molecule that work as an antioxidant has cycle or ring, unless it has another mechanism work in, like GSH (disulfide bridge).

Oxidative stress in neurodegenerative diseases:

1- Alzheimer's disease (AD): the most common neurodegenerative disease, characterized by aggregation of amyloid and tau tangles within neural tissues.

AD show evidence of ROS mediated-injury, as result increase in levels of malondyaldehyde and 4-hydroxynonenal within brain and cerebrospinal fluid. Increase the level of protein carbonyl moieties and hydroxylated guanosine as a marker of RNA and DNA damage by oxidative stress.

2- Parkinson's disease : second most common disease in neurodegenerative disease

Same story high levels of oxidative stress affecting protein , DNA and RNA (increased level of hydroxylated guanosine) , increased level of lipid peroxidation markers like malondialdehyde and 4-hydroxynonenal due to oxidative stress.

The marker of Parkinson's disease is aggregation of the alpha synuclein protein.

Mitochondria are dysfunctional in these diseases, the marker of mitochondrial dysfunction:

High level of oxidative stress, reduced ATP production, dysregulation of calcium, the permeability of pores in mitochondria is also defective producing all ROS to the cytoplasm, any of them is shown to be defective within neurodegenerative diseases.

**These are the results of many researches to ensure the relation between high levels of oxidative stress and neurodegenerative diseases:

1. Reduction in complex 1 activity ,they did an experimental study on mice ,they used MPTP-tetraphylla- material, its active form is MPP, this MPTP is similar to rotenone-which is complex1 inhibitor- ,if you inhibit complex1 or 2or 3or 4>> you're decreasing the movement of e->>less oxygen is used ,so high level of oxidative stress within the blood. When they gave MPTP to mice, they noticed Parkinsonian changes in mice. So it produces Parkinson's disease (PD),at the same time it works as ROS producer; because it inhibits complex1 so inhibition of mitochondria and as a result fragmentation of mitochondria by ROS.

2- They found genes related to Parkinson, mitochondria and formation of ROS all together such as: PINK1, DJ1 and Parkin. PINK1 is mitochondrial kinase, mutation to this gene cause a recessive form of Parkinson's disease. Also it's related to mitochondria so defect in this gene stop the function of mitochondria producing ROS at the same time produce Parkinson's disease.

3-in Alzheimer's disease (AD): reduction in complex4 activity in mitochondria from the hippocampus. Also, deregulation of calcium homeostasis.

Calcium enhances the enzymes involved in energy metabolism; enhance the work of the enzymes in Krebs cycle and Electron Transport Chain enzymes-activator of these enzymes-.

Simply it's an <activator> which will increase the movement of e- and pumping of protons in the ETC, then shutoff the ETC, at the end increasing the potential.

In addition, they found βA interacts with cyclophilin D-it's a part of PTP component-. This PTP is responsible for getting the materials out, so dysregulation of mitochondria resulting in degradation and phagocytosis of it.

So, there are main markers and evidences that the ROS have an effect on AD and PD, accordingly, anti oxidant treatment can reverse the case or stop the disease-modify it-. Antioxidants such as vitamin C, E and coenzyme Q, all contain a ring structure.

Is it promising or not?

Vitamin E supplementation in AD mouse model resulted in improved cognition and reduced βA deposition. However, vitamin C supplementation significantly reduced memory deficits in AD. Coenzyme Q has protective effects: decrease the neuronal damage and lessen the aggregation of α-synuclein protein.

All these experiments have been done on animals. But, the clinical trials on humans were ineffective in regard to the usage of vitamin C,E. Coenzyme Q attenuates the disease ,because it fastens the ETC as a part of ETC not as an antioxidant.

The results on animal model and on cells were effective, but the clinical trials on human were ineffective.

Why is it ineffective?

1-BBB. You can't deliver the drug to the site of action in high concentrations due to blood brain barrier.

2-Pathogenesis of disease could be different a little bit in humans than animals.

3- Biochemical rxns.

4- Oxidative stress needs a long period of time to be built-up. In mice model, they induce oxidative stress over a month to observe the changes. But within our bodies it needs 40 -50y to be built-up. So, TIME is a limiting factor.

Antioxidant treatment should be given early to stop the changes.

4- The producers of oxidative stress are multi in the nervous system so you should shutdown all ROS producers each one separately at the same time simultaneously to have an effect.

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