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Free radicals, oxidative stress and the Pandora box

Radicales libres, estrés oxidativo y la caja de Pandora

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Free radicals are atoms or groups of atoms that have a missing electron, so they are very reactive, trying to capture an electron from other atoms in order to achieve their electrochemical stability. The term «free radical» emphasizes a higher reactivity compared to molecules whose atoms are linked to each other by covalence (bond by electron sharing). Once the free radical has managed to subtract the electron (reduction) that it needs to stabilize, the stable molecule that loses it (oxidation) becomes in turn a free radical (it is left with an unpaired electron), thus initiating a chain reaction.

Because reactive species do not have specific acceptors/receptors, they have an indiscriminate aggressive capacity to interact with and damage cells and tissues.

Free radicals were first described in 1900, with the decomposition of hexa-phenylethane into two triphenylmethyl radicals. In 1956 Denham Harman hypothesized that oxygen radicals could be formed as products of enzymatic reactions *in vivo* and described free radicals as «Pandora's box of evils». He postulated the theory of free radicals causing aging, based on the premise that a single and common process (toxicity of free radicals) that is modifiable (increased) by genetic and environmental factors was responsible for the aging and death of all living beings.

There are three mechanisms of free radical formation:

1. Electronic transfer, in which the transfer of an electron to a molecule occurs.
2. Loss of a proton from a molecule.
3. Homolytic breaking of a covalent bond, so that each fragment conserves one of the paired electrons of the bond.

In cells, free radicals are produced through electron transfer reactions, with or without enzymatic participation.

As a product of metabolism, different types of free radicals are generated, such as:

1. Reactive Oxygen Species (ROS) examples of which are: superoxide anion, peroxide anion, perhydroxyl radical, hydroxyl radical.
2. Reactive Nitrogen Species (RNS): nitric oxide, peroxynitrite, among others.

The main sources of reactive species in the human body are the mitochondria, lysosomes, peroxisomes, as well as nuclear, cytoplasmic, and endoplasmic reticulum membranes.

Free radicals are also generated by several external factors, examples of which are: environmental pollution, exposure to ionizing radiation, tobacco consumption, chemical additives in processed foods and some xenobiotics such as pesticides, herbicides, and fungicides.

Superoxide anion ($O_{2^-}^{\bullet}$) is the first free radical generated. This ROS induced the formation of other reactive species in the vascular endothelium, such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH*), and peroxynitrite (ONOO−). Superoxide is generated through the partial reduction of molecular oxygen by the mitochondrial electron transport chain (ETC), as well as by NADPH oxidases, uncoupled endothelial nitric oxide synthase (eNOS) and xanthine oxidase.

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More than 95% of the O_2 consumed by the cells is reduced by the aqueous route to H_2O during mitochondrial respiration, while a small percentage (< 5%) is converted to superoxide. Once the free radical has been formed in the initial reaction, it has the ability to give up the electron to any other compound, originating new radicals, which constitutes the chain propagation reaction that can be amplified resulting in damage to body tissues. Superoxide anion is essentially produced in the mitochondria at the level of complex I and III, and its production is related to the leakage of electrons from the ETC that causes the partial reduction of molecular oxygen to O_{2}-* instead of water. ROS produced in the mitochondria can directly affect the functionality of the ETC complexes by oxidizing their iron-sulfur centers, thus exacerbating ROS production.

The NADPH oxidases (NOX), a family of membrane-bound enzyme complexes, catalyze the reduction of molecular oxygen to O_{2}-* by using NADPH as electron donor, this reflects their specific role in inducing a burst of ROS and bacterial killing in macrophages and other phagocyte cell types. NOX4 isoform is the most abundant in endothelial cells. Under physiological conditions, the NOX enzymes produce moderate levels of ROS that are required for normal redox signaling. In particular, the NOX2 and NOX4 isoforms induce proliferation and survival of endothelial cells activating MAPK and Akt. However, under pathological conditions, NOX4 may promote the formation of a pro-thrombogenic endothelial phenotype.

Nitric oxide synthases (NOS) are a family of enzymes that catalyze the production of NO and citrulline from oxygen and L-arginine. Electrons are transferred from NADPH to the heme iron and the cofactor tetrahydrobiopterin (BH4) to reduce and incorporate O_2 into L-arginine generating nitric oxide (NO) and citrulline. Three isoforms of NOS exist; neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial (eNOS). eNOS is the most abundant isoform expressed in endothelium. NO is a key determinant for vascular homeostasis since is the main vasodilatory substance release by the endothelium. However, under limited availability of substrates and/or cofactors, eNOS can generate superoxide instead of NO, a condition known as «uncoupling». Additionally, NO can react with superoxide generating peroxynitrite, another potent oxidant. Excessive peroxynitrite generation induces protein nitration contributing to mitochondrial dysfunction and endothelial cell dysfunction. Reduced BH4 bioavailability seems to be the main cause of eNOS uncoupling. In ROS-mediated endothelial dysfunction, BH4 is oxidized to dihydrobiopterin (BH2) that cannot function as a cofactor of eNOS leading to eNOS uncoupling. NOS uncoupling could also be induced by the endogenous competitive inhibitor asymmetric dimethylarginine (ADMA).

On the other hand, xanthine oxidoreductase (XOR) a key enzyme involved in purine degradation that catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid, is expressed as a dehydrogenase form (XDH) but, under inflammatory conditions, a switch from the reductase form to the oxidase form (XO). ROS produced by the XO enzyme are the major source of oxidative stress under ischemia/reperfusion injury. XO is involved in the increased ROS levels and vascular injury observed in diabetes.

Interestingly, under normal physiological conditions, the body neutralizes ROS through various antioxidant mechanisms. When the capacity of antioxidant substances is exceeded, a situation known as oxidative stress (OS) is established. In other words, OS is a condition that manifests when the production of highly reactive substances exceeds antioxidant mechanisms. OS is related to numerous diseases such as obesity, cancer, type 2 diabetes mellitus and cardiovascular disorders.

Essentially, antioxidant defenses are divided into two large groups: enzymatic and non-enzymatic; the first group refers to enzymes that constitute the first line of cellular defense against oxidative damage and these provide a protective function against biological oxidants, decreasing the intracellular concentration of free radicals. Among them are, superoxide dismutases (SOD), catalase, glutathione peroxidase (GPx), peroxiredoxins (Prx) and thioredoxin (Trx), among others. The non-enzymatic group, as a second line of defense, is made up of residual free radical scavengers, examples of them are:

reduced glutathione, uric acid, transferrin, lactoferrin, taurine, ceruloplasmin, ubiquinol, bilirubin, carotenoids such as vitamin A, vitamin E, vitamin C, butylhydroxytoluene (BHT), melatonin, among others.

Superoxide dismutase is the first in line among the enzymatic antioxidant defenses. Superoxide dismutases are a family of enzymes that catalyze the conversion of the superoxide anion to H_2O_2. There are three isoforms localized in different cellular compartments: 1) a cytosolic copper-zinc superoxide dismutase (SOD1 or CuZnSOD), 2) a predominantly mitochondrial manganese superoxide dismutase (SOD2 or MnSOD) and 3) an extracellular CuZnSOD (SOD3) with affinity for cell surface heparin sulfate proteoglycans. In endothelial cells the scavenging of superoxide by SOD1 and the H_2O_2 production has been related with the hyperpolarization factor showing the relevance of the enzymatic activity in the normal endothelial functionality.

H_2O_2 is reduced to molecular oxygen and water by antioxidant enzymes, including catalase and peroxidases. Catalase participates in the adaptive response of cells to oxidative stress, and its expression may be increased in endothelial cells by oxidative factors, such as oxLDL.

Glutathione peroxidase (GPx) catalyzes the reduction of H_2O_2 to molecular oxygen and water by using monomeric glutathione as electron donor. Oxidized glutathione is subsequently converted into the reduced form by glutathione reductase enzyme. Four different glutathione peroxidases have been identified in mammals, the most abundant expressed is the isoform 1 or GPx-1 this enzyme is located both in the mitochondria and cytoplasm of endothelial cells.

The peroxiredoxins (Prx) are thiol specific-enzymes that inactivate H_2O_2 to water using cysteine residues. Six peroxiredoxin isoforms have been identified, Prx1 isoform is implicated in the anti-oxidative and anti-inflammatory effects of laminar shear stress *in vitro*.

Thioredoxins (Trx) are located in both the cytosol and mitochondria. In addition to its role in regulating the redox state Trx is involved in the regulation of endothelial cell survival. In endothelial cells, Trx protein levels are finely regulated by H_2O_2 at low concentrations H_2O_2 increased Trx levels and protected cells from apoptosis, while at higher concentrations (100-500 μM) it induced apoptosis of endothelial cells via the degradation of Trx. These facts suggest that the modulation of reactive species may have several important roles in the cellular homeostasis.

Various pathological conditions, including hyperglycemia, hyperlipidemia, and arterial hypertension, as well as, aging and exposure to specific drugs, may influence endothelial function by disrupting the molecular mechanisms regulating NO bioavailability.

Free radicals and their role in disease showed that living organisms are not only adapted to a harmful coexistence with free radicals, but also have mechanisms developed for beneficial use of these. So, the production of reactive species or free radicals by itself is not harmful since they are participants in several normal functions oxidative stress is the condition that must be avoided or controlled.

There are numerous physiological functions that are modulated or controlled by signaling pathways related to redox-type reactions. It is known that the action of free radicals or their derivatives as physiological mediators includes: regulation of vascular tone, perception of oxygen pressure, regulation of functions that are controlled by oxygen concentration, as well as enhancing intracellular signal transduction of various membrane receptors, including the lymphocyte antigen receptor and oxidative stress responses that ensure the maintenance of the redox system (oxidation-reduction reactions).

A remarkable example is nitric oxide, this radical is a unique molecule, with the characteristics of a neurotransmitter; It has vasodilator activity, stimulates vascular smooth muscle synthesis and modulate platelet function. In the immune system, free radicals act as physiological mediators against bacterial infections.

Another function of free radicals is to be mediators in the synthesis of prostaglandins, cholesterol and steroid hormones. The hydroxylation of the aminoacids lysine and proline, necessary for collagen biosynthesis requires the participation of the free radical

• OH. However, while it is true that free radicals are fundamental elements in metabolism, they also constitute a risk, especially for cells and biomolecules, such as nucleic acids, proteins, polysaccharides and lipids.

Oxygen radical are capable of react with the nitrogenous bases or the pentoses that constitute DNA, forming the peroxyl radical, which results in structural damage and possible mutations. The oxidative damage to molecules is usually irreversible and can lead to denaturation of the molecule. In enzymes, it can impede their catalytic activity and in structural polysaccharides it causes their depolymerization, which leads to degenerative processes.

Lipids, especially those containing polyunsaturated fatty acids, are especially susceptible to developing uncontrolled oxidation processes. Cell damage is mainly caused by peroxidation of membrane lipids, allowing the passage of free radicals and calcium, which causes mitochondrial damage, releasing more free radicals into the intracellular environment, which cause a chain reaction, oxidizing proteins in their path, carbohydrates, membrane lipids (mitochondrial, nuclear and reticulum) including DNA itself.

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