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Hemoglobin, autoxidation, free radical generation, tissue injury and oxidative stress: An interesting correlation associated to the vascular accidents

Leonardo Marmo Moreira[1]* , Juliana Pereira Lyon[2]

[1]*Departamento de Zootecnia (DEZOO), Universidade Federal de São João Del Rei (UFSJ), São João Del Rei, Minas Gerais (MG), Brasil.*
[2]*Departamento de Ciências Naturais (DCNAT), Universidade Federal de São João Del Rei (UFSJ), São João Del Rei, Minas Gerais, Brasil.*
**Author for correspondence, E-mail: leonardomarmo@gmail.com*

Abstract. The present manuscript is focused on the relation between hemoglobin, autoxidation, free radical generation (with especial emphasis on reactive oxygen species (ROS) and reactive nitrogen species (RNS)), and the biochemical and physiological processes that occur in some biological and pathological situations, mainly vascular accidents, such as stroke and myocardial infarction. Indeed, several types of free radicals are quite associated with the chemical processes of circulatory accidents as well as the subsequent biological tissue lesions. Interestingly, the hemorrhagic and ischemic circulatory processes of vascular diseases are related to the presence of several free radical species. This work presents various correlations between these chemical and biochemical agents, favoring to understand the important role of the free radicals in this kind of disease. It is also suggested that the analysis of these chemical species could constitute a strategy to evaluate the evolution of the circulatory disease.

Keywords: Heme proteins; Reactive oxygen species (ROS), Reactive Nitrogen Species (RNS), stroke

Hemoglobina, auto oxidação, geração de radicais livres, lesão tecidual e estresse oxidativo: Interessante correlação associada aos acidentes vasculares

Resumo. O presente manuscrito é focado sobre a relação entre hemoglobina, auto oxidação, geração de radicais livres (com especial ênfase em espécies reativas de oxigênio (EROs) e espécies reativas de nitrogênio (ERNs)), e os processos bioquímicos e fisiológicos que ocorrem em algumas situações biológicas e patológicas, principalmente acidentes vasculares, tais como acidente vascular encefálico (“derrame”) e infarto do miocárdio (infarto do coração). De fato, os diversos tipos de radicais livres são completamente associados aos processos químicos dos acidentes circulatórios assim como as subsequentes lesões nos tecidos biológicos. Interessantemente, os acidentes circulatórios hemorrágico e isquêmico das doenças vasculares estão relacionadas à presença de diversas espécies de radicais livres. Este trabalho apresenta várias correlações entre estes agentes químicos e bioquímicos, favorecendo compreender o importante papel dos radicais livres neste tipo de doença. É também sugerido que a análise dessas espécies químicas poderia constituir uma estratégia para avaliar a evolução da doença circulatória.

Palavras chaves: Hemoproteínas, espécies reativas de oxigênio (EROs), espécies reativas de nitrogênio (ERNs), acidente vascular encefálico (“derrame”)

Hemoglobina, autooxidación, generación de radicales libres, daño tisular y estrés oxidativo: correlación interesante asociada con derrames

Resumen. El presente manuscrito se centra en la relación entre la hemoglobina, la autooxidación, la generación de radicales libres (con especial énfasis en las especies reactivas de oxígeno (ROS) y las especies reactivas de nitrógeno (ARN)), y los procesos bioquímicos y fisiológicos que ocurren en algunas situaciones biológicas y patológicas, principalmente accidentes vasculares, como accidente cerebrovascular ("Derrame") e infarto de miocardio (infarto de corazón). De hecho, los diversos tipos de radicales libres están completamente asociados con los procesos químicos de los accidentes circulatorios, así como las lesiones posteriores a los tejidos biológicos. Curiosamente, los accidentes circulatorios hemorrágicos e isquémicos en enfermedades vasculares están relacionados con la presencia de varias especies de radicales libres. Este trabajo presenta varias correlaciones entre estos agentes químicos y bioquímicos, favoreciendo la comprensión del importante papel de los radicales libres en este tipo de enfermedad. También se sugiere que el análisis de estas especies químicas podría constituir una estrategia para evaluar la evolución de la enfermedad circulatoria.

Palabras claves: Hemoproteínas, especies reactivas de oxígeno, especies de nitrógeno reactivo, derrame cerebral

Introduction

The tendency to the oxidation is one of the most determinant properties of heme proteins. The difficulty to remain in its ferrous state (Fe^{II}), which is the functional oxidation state of heme group of carrier heme proteins, such as hemoglobin, is associated to several problems, such as ferric anemia, which is caused by excess of the well-known ferric-hemoglobin or met-hemoglobin. The process known as autoxidation is inherent to the physico-chemical properties of the heme groups in heme proteins, which makes the hydrophobic isolation of the heme a determinant characteristic to hemoglobin to maintain its native conditions. On the other hand, the tendency to autoxidation, which is a general property, presents different degree in each distinct hemoglobin.

The properties of hemoglobin are very relevant to several species, including some that do not produce this heme protein. Indeed, hemoglobin-binding proteins have been identified in a significant number of pathogens, indicating that the selective recognition of this heme protein can happen at the cell surface (Sengupta et al., 1999). It occurs due to different objectives, such as inorganic iron acquisition (Pendrak et al., 2004).

Several vascular accidents can increase the oxidative processes, originating additional problems associated to circulatory lesions. Mori et al. (2004) claim that the iron released from hemoglobin, and hemoglobin itself in processes as head injuries or hemorrhagic mechanisms, which are associate to extravasation of blood and breakdown of red blood cells and hemoglobin, can generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mori et al., 2004). In fact, various examples of ROS, such as superoxide anion, hydroxyl radical, hydroperoxide, hydrogen peroxide, between others, are associated to various degenerative processes (Minetti et al., 1993).

There is a growing interest in the role of oxygen-free radicals, which are usually called “reactive oxygen species” (ROS), and “reactive nitrogen species” (RNS), in experimental and clinical medicine, between other areas. ROS/RNS are known due to harmful or beneficial actions in living systems (Valko et al., 2006). In the beneficial effects, it is possible to mention the physiological roles in cellular responses to different pathological processes, such as in the defense against infectious agents and the cellular signaling systems, while in the harmful ones are associated to oxidative damages upon DNA, proteins, lipids, which would be associated to several age-dependent diseases like, for example, cancer, arteriosclerosis, arthritis, neurodegenerative disorders, between others, in spite of the counteract of antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes (Valko et al., 2006).

The reaction of free radicals with hemoglobin destabilizes the chemical constitution and the spatial arrangement of these proteins, originating the species called hemi chrome (the iron-porphyrin is coordinated by two histidine (distal and proximal histidine). Considering intracellular hemoglobin, such

as the human hemoglobin, the hemi chrome formation would be associated to the formation of the Heinz bodies (Minetti et al., 1993). Heinz bodies, which can be observed in senescent red blood cells, unstable hemoglobin and drug-induced hemolytic anemias, interact with the cell membranes and alter cell properties, contributing to premature red cell death (Minetti et al., 1993). It is important to notice that the free radicals generate hemoglobin degenerations as well as the hemoglobin autoxidation and/or blood extravasation tends to originate free radicals.

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons frequently furnishes a significant degree of chemical reactivity to a free radical. In this context, the free radicals derived from oxygen are considered the main group of free radicals (Valko et al., 2006). In any case, it is also important to mention the great number of relevant reactive nitrogen species (RNS) in biological media. ROS can be produced from endogenous and exogenous sources. Potential endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation (Valko et al., 2006). Furthermore, oxidative processes also occur due to pharmacological actions of several drugs. In this context, the red blood cells are studied as a source of free radicals and as a target for oxidative damage. In fact, a wide variety of drugs and xenobiotics, which can undergo oxidation-reduction reactions, provokes red cell destruction and hemolytic anemia (Winterbourn, 1985). It is important to notice that the interaction between the xenobiotic and hemoglobin is relevant in the process, which is frequently characterized by ferric heme generation, and formation within the red cells of Heinz bodies, inclusions of denatured and precipitated hemoglobin (Winterbourn, 1985).

This point is very relevant, since the oxidation of hemoglobin is deeply associated to the loss of native tridimensional configuration of the heme proteins. Consequently, this process is related to denaturation and, in some cases, precipitation of different types of heme proteins, including extracellular and cellular hemoglobin. Indeed, in some species the hemoglobin is not associated to red blood cells, being known as extracellular hemoglobin.

In the case of the cellular hemoglobin (differently of the extracellular hemoglobin, which are found in several animal species, such as the worms named *Lumbricus terrestris* and *Glossoscolex paulistus*), the formation of Heinz bodies is a consequence of the denaturation and precipitation of hemoglobin in the red blood cells.

The generation of free radicals and/or ROS and RNS tends to favor a higher impact related to the rupture of the blood vessels. The volume of blood is associated to various problems, such as edema, increase in the intracranial pressing, inflammation and secondary lesions. In this way, the presence of great number of chemical species with great reactive potential, mainly in redox reactions, would favor a higher local impact and, eventually, more significant systemic consequences, considering the great number of biomolecules that can be reacted, mainly oxidized, losing biochemical functions.

The above-mentioned process, probably, is associated to the autoxidation of the hemoglobin inserted in the red blood cells of the extravasated blood. In fact, the human blood is not very resistant to the autoxidation in “non-protected media”, implying that the release of hemoglobin in an external medium to the red blood cells must favor the attack of Lewis`s bases, which can act as nucleophiles to the heme ferrous center. This process favors the generation of an intermediary heptacoordinate heme species, which favors, in an intense way, the release of the superoxide anionic radical (which consists in a reactive oxygen species ROS, producing, concomitantly, a ferric species that can carry oxygen molecules.

This discussion is significantly different when compared with the studies focused on oxygen saturation (Dziedzic & Vidyasagar, 1989; Ferreira & Bandeira, 2003; Granzoto, 1989; Vijayakumar et al., 1997). Indeed, the oxygen ligand (oxygen molecule) does not leave the first coordination sphere of the metallic center (ferrous ion) in its molecular form (oxidation state zero), but it is releases as a radical anion, which, in this case, is named superoxide radical anion, in a more complex phenomenon called “autoxidation”. This name, autoxidation, is used because the oxygen molecule coordinated to the porphyrin ferrous center (Fe^{II}-O_2) is responsible for the oxidation of the ferrous ion Fe^{II} to ferric ion Fe^{III}, leaving the porphyrin as O_2^- (superoxide anion). In this way, a series of circulatory problems generated subsequently to the extravasation of blood and breakdown of red blood cells and hemoglobin

would occur not only due to the ischemic processes and increase in the intracranial pression. They would be also caused due to the intense free radical generation.

The release of free radicals can provoke a significant oxidative stress, which can produce a severe lesion in the respective biological tissue. Therefore, the original redox process, which is the autoxidation, originates various following redox processes, which can provoke complex injuries in the tissue that is associated to the circulatory accident.

Furthermore, the great number of ROS and, in a second moment, RNS, can provoke "secondary or indirect consequences". It occurs because free radicals can be reabsorbed to the circulation and, through the vascular system, reach biological tissues that are more distant of the region of the circulatory accident. This subsequent process depends of the stability of the ROS and/or RNS, i. e., it is associated to the half-time life of the free radicals.

Consequently, the physiological impact of the vascular accident can reach not only the region affected by the trauma, but equally other tissues, due to the free radicals that was reabsorbed in the systemic circulation.

It is possible that the local and systemic impacts of this process can be attenuated by the presence of natural antioxidants, such as vitamin E and vitamin C (Mori et al., 2004), which can minimize the occurrence of post traumatic implications as function of the attenuation of the oxidative stress. This attenuation is generated by the electronic reduction of the several free radicals generated in the biological medium. Indeed, the reductase enzymes did not avoid a significant oxidative stress. In this way, the biological reduction by some biomolecules with low redox potential, such as vitamins, can minimize several pathological consequences. Thus, depending of the quantity and the oxidant power of the free radicals as well as the tissue reached by the radicals, it is possible to identify a higher or lower effective pathologic impact.

In this context, the presence of natural antioxidants in the tissue affected by the trauma as well as the tissue that was reached, in a second moment by the free radicals, via systemic circulation, seems to be decisive to determine the intensity of the local and/or systemic pathological consequences.

Free radical generation in stroke and myocardial infarction

The molecular mechanisms are not still totally understood, but there are evidences that ROS and RNS are important factors in the actions that injuries the cerebral tissue (Cossentini et al., 2019). In this way, mainly in the hemorrhagic occurrences, the presence of ROS and RNS constitute one of the most important factors that cause tissue lesions.

The reactive species have significant effect upon the destruction and necrosis of cells, which includes lipid peroxidation, protein denaturation and enzyme inactivation, DNA injuries, Ca^{2+} release and injuries to the cytoskeleton. The cells present mechanisms to eliminate the toxic products, but the biological environment of the stroke is not sufficient to manage the oxidative charge, even with the increase of reductor enzymes, which can provoke the initial steps of necrosis and apoptosis (Cossentini et al., 2019).

On the other hand, in the ischemia, the increase of the hypoxic process favors the lactate increase, pH decreases and proteolytic enzymes increase. When reperfusion occurs, the return of oxygenation, in some cases, is not enough to the reach the normal conditions of cellular physiology. It occurs due to the great intracellular calcium concentration; the presence of ROS and RNS, generated by ischemia; and the toxic actions of substances originated from metabolism of fatty acids, which affect the cellular mechanisms (Rodrigues et al., 2016). Indeed, even in the coronary disease, it is probable that the ischemic process induces the acid release, such as lactic acid, by the cardiac muscle, or other products that promote pain, such as histamine and proteolytic enzymes, which are not removed more fast by the blood that is in a slow movement (Guyton & Hall, 2011).

The relevance of free radicals to the diagnosis and treatment of circulatory accidents

Frequently, the diagnosis of circulatory accidents, such as stroke, is a not trivial task in several situations (Valente et al., 2012). The detection of free radicals could be a novel strategy to contribute to

the identification and characterization of this kind of disease. Furthermore, the control of the free radicals' level can be an indicative of some steps of the biological tissue recovery.

Conclusions

The understanding of the role of free radicals in circulatory diseases is a relevant topic to several areas, such as biochemistry, pharmacology, human medicine and veterinary medicine. Indeed, free radicals are important agents of several tissue injuries. Furthermore, free radicals could be considered potential sensors or labels of several biochemical and physiological processes, which could be applied to diagnosis and treatment of several diseases in human and veterinary medicines.

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