Opinion

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.49.007833

Measuring ROS and Oxidative Damage in Human Health: Relevance and Methodological Considerations

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ARTICLE INFO

Received: March 28, 2023

Published: April 10, 2023

Citation: Alvaro Becerra Farfan. Measuring ROS and Oxidative Damage in Human Health: Relevance and Methodological Considerations. Biomed J Sci & Tech Res 49(4)-2023. BJSTR. MS.ID.007833.

ABSTRACT

Reactive oxygen species (ROS) are a wide variety of oxidative molecules with relevant biological properties and functions. A redox imbalance, favouring a sustained increase in ROS, generally leads to oxidative damage (OD); associated with the onset, progression, and severity of various pathologies and ageing. This strategic biological importance makes them relevant biomarkers in both health and disease. However, the literature describes multiple difficulties, errors, and methodological restrictions in measuring ROS and OD; to use them as biomarkers reliably.

Keywords: Reactive Oxygen Species; Oxidative Damage; Biomarker; Inflammation

Introduction

Reactive oxygen species (ROS) are by-products of normal aerobic cellular metabolism, which are relatively reactive, partially reduced, and play important physiological roles in maintaining homeostasis. Usually, a redox balance allows the body to respond adequately to physiological needs. Whereas the result of a sustained and severe increase in ROS, under conditions of oxidative stress, promotes OD to biomolecules (e.g., proteins, lipids, polysaccharides, and nucleic acids) leading to cell deterioration and death [1] Accumulated OD expressed in an inflammatory phenotype is critical in the onset and development of neurodegenerative, cardiovascular, and renal diseases, some cancers, diabetes, infertility and ageing. Thus, ROS and OD measurements have become strategic tools to assess, among others: physiological redox status, subclinical symptoms, disease status and progression, and to record possible beneficial effects of antioxidants [2,3]. However, several researchers have reported problems, limitations and difficulties in measurements. [4-6] Inadequate use of terminology, lack of knowledge of the pathways or molecular interactions involved, and incorrect choice of assays or measurement protocols; are some of the most frequently reported shortcomings. Despite the solid knowledge of redox biology, it can often lead to confusion and loss of validity in biomarker measurements. This article aims to discuss the relevance and methodological considerations of measuring ROS and oxidative damage in human health.

Discussion

The critical role of ROS and the consequent OD in the gestation, development and severity of many diseases and ageing, has led to the development and validation of new and improved protocols, commercial kits and methods for their use as biomarkers. By definition, a biomarker is any substance that can describe or measure a biological system's characteristics. However, for a biomarker to present consensus and validation, it must be measured accurately and reproducibly [7] The relatively reactive and short half-life nature of oxidized species or products in biological systems presents a significant theoretical/technical challenge in their direct measurement and in determining their potential biological effects.

This fact often results in measurement inaccuracies or errors and possible misleading claims, which hinders the proper advancement of knowledge of redox biology. For example, some clinical studies have sought to measure the beneficial effect of antioxidant intake by measuring non-enzymatic antioxidant capacity (NEAC), but the different methods used in the investigations show different results [8] To address the aforementioned situation, several interdisciplinary efforts worldwide seek to improve the nomenclature, measurements of ROS/oxidized products and possible signalling pathways or biological effects, to generate new guidelines and consensus, contributing to providing a greater biological validity to these tools and their use as biomarkers.

Thus, some relevant aspects that should be considered when measuring ROS and OD are the following:

I. New research suggests using assays that allow identification of the reactive species individually and specifically (e.g., $O_2^{\bullet-}$ and H_2O_2) in favour of withdrawing of general or unspecific concepts of «ROS» or «oxidative stress», along with understanding oxidative damage; through signalling pathways and products generated.

II. Employ more than one assay to directly and specifically measure the species under study. Additionally, use complementary assays to assess and quantify possible oxidative (e.g., lipid peroxidation products) and inflammatory damage in the system under study.

III. Review and pay attention to common methodological steps: Sample stability during storage and preparation. Use adjusted controls and analyses; consider that they measure oxidative modifications influenced by environmental oxygen.

IV. Use absolute values when publishing results (e.g., molar concentration); to facilitate comparison between studies.

The following previous reviews [9,10] are recommended to learn more about the topic.

Undoubtedly, the proper use of terminology and the development of more accurate measurement methods (e.g., real-time) will help us better understand specific molecular mechanisms, contributing to validating redox biomarker's use in both health and disease, and transforming them into fundamental health tools.

Acknowledgment

The author thank Dr. Manuel E. Cortés for the English language review and insightful comments of the manuscript.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.49.007833

Alvaro Becerra Farfan. Biomed J Sci & Tech Res