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Chapter

Reactive Oxygen Species (ROS) in the Pathophysiology of Rheumatoid Arthritis (RA)

Haseeb Ahsan, Mohammad Yusuf Hasan and Rizwan Ahmad

Abstract

Free radicals are highly reactive molecules that are unstable and have extremely short-half-life. They are derived from either oxygen (reactive oxygen species, ROS) or nitrogen (reactive nitrogen species, RNS) in mitochondria, plasma membrane and endoplasmic reticulum due to oxidative stress and damage. ROS/RNS are physiologically useful at low concentrations and are responsible for the activation of redox-sensitive signaling pathways, phagocytosis of infected cells and removal of abnormal and aging cells. The endogenous sources of ROS are the electron transport chain, the respiratory burst of phagocytes and oxidation of lipids. These radicals react with biomolecules such as DNA, proteins and lipids and may cause pathophysiological conditions such as autoimmunity, carcinogenesis, and neurodegenerative diseases. The role of ROS in autoimmune response remains complex and they have been implicated in the initiation, generation and amplification of novel epitopes. ROS also appears to play a critical role in rheumatoid arthritis (RA), a systemic autoimmune disease of the joints also known as inflammatory arthritis (IA). ROS are involved in the initiation of various signaling pathways and have a significant role in the pathophysiology of RA.

Keywords: ROS, RNS, RA, autoimmunity, free radicals, rheumatoid arthritis

1. Introduction

A free radical is a “molecule containing one or more unpaired electron(s) and which is capable of independent existence”. Free radicals are highly reactive species and are involved in several metabolic processes including oxidative reactions in mitochondria, and ‘oxidative burst’ of phagocytes, etc. In excess, free radicals lead to diseases including autoimmune, cardiovascular, neurodegenerative, cancers and must be reduced to minimize these pathological conditions. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two types of free radicals formed in the body and consist of both radical and non-radical moieties [1]. Under normal conditions, living organisms utilize respiration to survive, they undergo a process of reduction of oxygen molecules through the addition of four electrons resulting in the formation of water. This process produces molecules such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) as a byproduct. During energy

transduction via electron transport of molecular oxygen, $O_2\text{---}\cdot$ is formed intracellularly within the mitochondria, which may potentially lead to the development of a variety of pathophysiological conditions. [2, 3] (**Figure 1**).

The $O_2\text{---}\cdot$ is converted into stable non-radical H_2O_2 by the enzyme superoxide dismutase (SOD). Catalase (CAT) and glutathione peroxidase (GPx) convert H_2O_2 to H_2O and O_2 . Further, H_2O_2 is converted by myeloperoxidase (MPO) in the neutrophils to hypochlorous acid (HOCl), which is a strong oxidant acting as a bactericidal agent in phagocytic cells. The reaction of HOCl with H_2O_2 yields singlet oxygen (1O_2) and water. Finally, H_2O_2 is converted in a spontaneous reaction catalyzed by Fe^{2+} (Fenton reaction) to the highly reactive $\cdot OH$ (**Table 1**). The $\cdot OH$ causes oxidative damage to biological molecules such as lipid, protein and DNA leading to the etiopathogenesis of autoimmune disorders [5]. The excess generation of $OH\cdot$ and peroxyxynitrite ($ONOO\text{---}$) causes oxidative damage to cell membranes and lipoproteins leading to lipid peroxidation resulting in the formation of harmful compounds such as malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) [6]. ROS/RNS damage nucleic acids leading to change in the function and conformation of DNA resulting in the formation of strand breaks, nitrogenous base damage-causing mutations [1]. The ROS are also produced from other sources such as NADPH oxidase (NOX) inactivated phagocytes and endothelial cells, macrophage and polymorphonuclear cells (PMN), lysosome and microsomes. ROS is also involved in inflammatory reactions through the activation of nuclear transcription factor kappa B (NF- κB), leading to upregulation of pro-inflammatory cytokines and leukocyte adhesion molecules (LAM) [5].

Oxidative stress results from reactive species from the biochemical reactions within the body including NADPH oxidases (NOXs), nitric oxide synthase (NOS), nitrate reductase (NR), mitochondrial electron transport chain (ETC) and the hydrogen sulphide (H_2S) producing enzymes cystathionine- β synthase (CBS) and cystathionine- γ lyase (CSE). Superoxide undergoes a dismutation reaction to generate H_2O_2 which, in the presence of transition metal ions (e.g., ferrous ions), forms the $\cdot OH$. MPO, produces HOCl MPO is a heme-containing peroxidase expressed mainly in neutrophils and to a lesser degree in monocytes. MPO, in the presence of H_2O_2 and halides, catalyzes the

Figure 1. Pathways of ROS/RNS in the body. NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; GSH, glutathione; GSSG; glutathione disulfide; SOD, superoxide dismutase; NOS, nitric oxide synthases; MAO, monoamine oxidase; MPO, myeloperoxidase (reprinted from [3]).

Equations	
Oxygen radical generation	
NADPH oxidase:	$2O_2 + NADPH \rightarrow 2O_2^{\cdot-} \text{ (superoxide)} + NADPH^+ + H^+$
Spontaneous conversion:	$2O_2^{\cdot-} + 2H^+ \rightarrow [2HO_2^{\cdot} \text{ (hydroperoxyl radical)}] \rightarrow O_2 + H_2O_2$
Superoxide dismutase:	$2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$
Myeloperoxidase:	$Cl^- + H_2O_2 \rightarrow OCl^- \text{ (oxidised halide)} + H_2O$
Reactive oxygen species secondary products	
	$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\cdot} \text{ (hydroxyl radical)} + Fe^{3+}$
	$Fe^{3+} + O_2^{\cdot-} \rightarrow O_2 + Fe^{2+}$
	$O_2^{\cdot-} + HOCl \rightarrow O_2 + OH^{\cdot} + Cl^-$
	$H_2O_2 + OCl^- \rightarrow {}^1O_2 \text{ (singlet oxygen)} + H_2O + Cl^-$
	$NH_3 + HOCl \rightarrow NH_2Cl \text{ (chloramine)} + H_2O$
	$ \begin{array}{c} R - CHNH_2 - COOH \\ \text{(amino acids)} \end{array} + HOCl \rightarrow \begin{array}{c} R - CHNHCl - COOH \\ \text{(chloramines)} \end{array} + H_2O $ $ \begin{array}{c} R - CHO + CO_2 + NH_4^+ + Cl^- \\ \text{(aldehydes)} \end{array} $
Nitrogen radical generation and secondary reactions	
Nitric oxide synthetase:	$\text{arginine} + O_2 + NADPH \rightarrow NO^{\cdot} + \text{citrulline} + NADP^+$
	$NO^{\cdot} + O_2^{\cdot-} \rightarrow ONOO^- \text{ (peroxynitrite)}$
	$ONOO^- + H^+ \rightarrow ONOOH \text{ (peroxynitrous acid)}$
	$ONOOH \text{ (peroxynitrous acid)} \rightarrow NO_3^- \text{ (nitrate ion)}$
	$ONOOH \rightarrow NO_2^+ \text{ (nitronium ion)}$
	$ONOOH \rightarrow NO_2^{\cdot} \text{ (nitrogen dioxide radical)}$
	$ONOOH \rightarrow OH^-$
	$ONOOH \rightarrow OH^{\cdot} \text{ (hydroxyl radical)}$
Lipid peroxidation:	$LH + \text{Radical}^{\cdot} \rightarrow L^{\cdot} + RH$
	$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \text{ (lipid peroxy radical)}$
	$LH + LOO^{\cdot} \rightarrow L^{\cdot} + LOOH \text{ (leading to lipid propagation)}$ $LOO^{\cdot} + \text{TocOH } (\alpha - \text{tocopherol}) \rightarrow LOOH + \text{TocO}^{\cdot} \text{ (chain termination)}$

Table 1.
 Equations showing products generated by oxygen/nitrogen radicals (reprinted from [4]).

formation of ROS such as HOCl. The MPO/HOCl system plays an important role in microbial killing by neutrophils. Moreover, it has also also been shown to be a local mediator of tissue damage and the inflammation in various inflammatory diseases. When ROS production exceeds the physiological antioxidant defense, oxidative stress occurs consequently leading to oxidative modification of proteins. This protein alteration may lead to the formation of neoepitopes resulting in the formation of autoantibodies [7, 8]. The pathogenesis of autoimmune diseases (ADs) such as RA is

characterized by the loss of peripheral tolerance to autoantigens, excessive activation of T and B cells, which leads to increased levels of cytokines and autoantibodies (rheumatoid factor, RF; anti-cyclic citrullinated peptide antibodies, ACPA; etc.) [9].

Studies have shown that anti-carbamylated proteins anti-CarP and anti-type II collagen antibodies can serve as a promising diagnostic such diagnostic tool in such ADs. The activation of endogenous cellular antioxidant defense systems (e.g., nuclear erythroid 2-related factor 2; Nrf2-dependent pathway), inhibition of ROS/RNS source (e.g., isoform-specific NOX inhibitors), etc., may become the potential future strategies for redox-based therapeutic compounds [7].

2. Pathophysiology of free radicals

When there is an imbalance between free radical production and removal, causing oxidative and resulting in aberrant metabolic processes in the body. To neutralize and eradicate the free radicals, enzymatic and non-enzymatic antioxidants such as vitamin E (tocopherols and tocotrienols), SOD, CAT, GPx, vitamin C (ascorbic acid), beta-carotene (β -carotene), coenzyme Q10 (CoQ10), play an important role in the body's defense system against these toxic species. Thus, these biological molecules demonstrate an essential role in the quenching or removal of harmful free radicals. Antioxidants such as polyphenolic compounds can reduce the concentration of ROS by scavenging them, and hence potentially averting any deleterious oxidative damage to macromolecules [2]. Several degenerative conditions such as cardiovascular, neurological, diabetes, ischemia-reperfusion injury and aging have been shown to be caused by pathophysiology of free radicals. Autoimmune diseases (ADs) such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are prominent examples where free radical-induced damage occurs in cells and tissues [10]. The diseases, which are caused by free radical, are usually characterized into two categories: one relating to cancer and diabetes involving "mitochondrial oxidative stress" and occur due to impaired glucose tolerance while the second category involves inflammatory oxidative conditions resulting in atherosclerosis and chronic inflammation. Mutation in DNA, which more often occurs due to oxidative damage is strongly correlated to the etiology of cancer, and is one of the initiators of carcinogenesis in which elevated levels of DNA lesions become apparent in many tumors. ROS-induced changes are also found in various diseases of the heart such as cardiomyopathy, ischemic heart disease, hypertension, atherosclerosis, and heart failure [1]. Several studies have established the key role of oxidative stress in the pathology of the joints which includes inflammatory infiltration, synovial proliferation, and angiogenesis [11, 12].

3. Rheumatoid arthritis

RA is a systemic AD of the joints also known as inflammatory arthritis (IA), in which several additional organ systems are known to be involved, including the pulmonary, cardiovascular, ocular, and cutaneous systems. However, the presence of IA, defined as tenderness and swelling consistent with underlying synovitis is the hallmark for the clinical diagnosis of RA. It is characterized by persistent joint inflammation with accompanying bone and cartilage damage and the formation of autoantibodies [13] (**Figure 2**). Most of the RA patients have rheumatoid factor (RF, autoantibody against the Fc portion of IgG) and anti-citrullinated peptide antibodies (ACPA) [14–16]. The exact cause of RA

Figure 2.
a. Rheumatoid arthritis. The oxidative stress and local inflammatory mediators manifest mainly in the joint resulting in pain and pain and inflammation which may lead to and joint damage and disability at a later stage (reprinted and modified from [13]). b. Oxidative stress and inflammation in rheumatoid arthritis (reprinted and modified from [13]).

is still not completely understood, although there is a strong evidence of the genetic involvement in disease process. The class II major histocompatibility complex (MHC) molecules, HLA-DR1 and HLA-DR4 are regarded as the major genetic risk factors in the etiopathogenesis of RA [17–19].

Several studies suggest that oxidative stress leads to defective redox signaling and damage to biomolecules in the pathogenesis of RA [12]. The mutations in genes that

encode inflammatory and enzymatic molecules involved in the oxidative burst are responsible for the development of RA. In arthritic inflammation, ROS acts as an inflammatory mediator and contributes to destruction of collagen tissues and the overproduction of ROS is associated with damage leading to cartilage degradation. The damaged chondrocytes, which are essential for the structure and function of cartilage, are stimulated to undergo apoptosis. Fewer chondrocytes are unable to self-repair the cartilage and therefore promote the breakdown of the extracellular matrix in joints. Furthermore, H_2O_2 leads to chondrocyte lipid peroxidation which is also linked to protein oxidation and degradation of cartilage matrix [20].

Additionally, the functional genetic analysis showed that the single nucleotide polymorphisms (SNPs) in the neutrophil cytosolic factor 1 (Ncf1) coding region is associated with genetic susceptibility to RA [21]. The patients with chronic granulomatous disease (CGD) also have amplified susceptibility to RA [21, 22]. Furthermore, a study of NOX2-deficient mice revealed that the absence of ROS averts resistance to autoimmune arthritis. A collagen-induced arthritis model produced by Ncf1 mutation in mice has more acute symptoms, higher anti-CII IgG levels, and stronger T helper type 1 (Th1) responses than wild-type mice. Interestingly, the study also found that T cells from Ncf1-mutated mice reacted more actively to antigen presenting cells (APCs) [23]. Mice with mutated collagen (MMC) have increased resistance to arthritis mediated by a mutated immunodominant epitope in collagen type II that binds to the MHC class II molecule. When these MMC mice are bred with NOX2-deficient mice, their immune tolerance to arthritis disappears, and they display increased autoimmune T cell and higher levels of anti-CII IgG levels [24]. Therefore, the pathophysiology of ROS in RA is an imbalance in homeostasis between pro- and antioxidative and pro and anti-inflammatory conditions, which may lead to the damage of connective tissues, multiple joints and other organs in the body.

4. Conclusion

In ADs, the immune system recognizes and attacks its tissues i.e., self-destruction. Activation, proliferation, and apoptosis of immune cells are dependent on the controlled production of ROS. However, under chronic inflammatory conditions, large amounts of ROS generated are responsible for the pathophysiology of many human disorders. A characteristic feature of ADs is the presence of autoantibodies and inflammatory conditions and cells such as mononuclear phagocytic system, autoreactive T lymphocytes and autoantibody-producing B cells (plasma cells). RA is a systemic autoimmune disease of the joints with underlying synovitis and is also known as inflammatory arthritis. Increased apoptosis and decreased clearance of apoptotic cells observed in systemic autoimmunity may be a contributing factors in autoimmune disorders such as RA and SLE. Since ROS have been implicated in the initiation and progression of autoimmunity, their role in autoimmunity remains complex. The pathophysiology of ROS in RA though not well understood but could be understood as an imbalance in homeostasis between pro- and antioxidant conditions and pro and anti-inflammatory states, which may become chronic leading to damage of connective tissue and multiple joints and other organs in the body. Moreover, it is important to consider the therapeutic development of antioxidants and selective ROS inhibitors as a tool that could be used in the prevention and treatment of a broad range of ADs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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