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Review Article

Macrophages in oxidative stress and models to evaluate the antioxidant function of dietary natural compounds

Omir Adrian Castaneda [a], *Sheng-Chi Lee* [b], *Chi-Tang Ho* [c], *Tzou-Chi Huang* [d,*]

[a] *Department of Food Science, National Pingtung University of Science and Technology, Pingtung, Taiwan*
[b] *Department of Orthopaedics, Pingtung Branch, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan*
[c] *Department of Food Science, Rutgers University, New Brunswick, NJ, USA*
[d] *Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan*

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ABSTRACT

Antioxidant testing of natural products has attracted increasing interest in recent years, mainly due to the fact that an antioxidant-rich diet might provide health benefits. Activated macrophages are a major source of reactive oxygen species, reactive nitrogen species, and peroxynitrite generated through the so-called *respiratory burst*. Constitutively released proinflammatory cytokine, especially tumor necrosis factor-α, triggers nuclear factor-κB, and activator protein-1 translocation leading to the over production of reactive oxygen species and reactive nitrogen species in macrophages. Activation of transcription factors in the long-lived tissue-resident macrophages and/or monocyte-derived macrophages, trigger epigenetic modifications leading to the pathogenesis of chronic diseases. Nutraceuticals including lipid raft structure disruption agent, cholesterol depletion agent, farnesyltransferase inhibitor, nuclear factor-κB blocker (α,β-unsaturated carbonyl compounds), glucocorticoid receptor agonist, and peroxisome proliferator-activated receptor-γ agonist have long been used to inactive macrophage. The inhibition effects on the formation of nitric oxide, superoxide, and nitrite peroxide may be responsible for the anti-inflammatory functionalities. Activated macrophage models could be used to identify the active components for functional diets development through a multiple targets strategy.

* *Corresponding author*. Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan.
E-mail address: tchuang@mail.npust.edu.tw (T.-C. Huang).

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1. Oxidative stress and chronic diseases

The operational definition of *oxidative stress* is given by Lushchak [1] as "a situation when steady-state reactive oxygen species (ROS) concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation, and damaging cellular constituents." Reactive free radicals, including superoxide, hydroxyl radical, and peroxyl radical, generally result in degradation of protein, lipid peroxidation, and oxidation of DNA, which have possible linkage with many chronic diseases, such as diabetes, cancers, and atherosclerosis. ROS play an important role related to the degenerative or pathological processes of various serious diseases, such as age-related diseases, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, cataracts, and inflammation [2].

1.1. *Reactive oxygen species*

ROS is a collective term that includes both oxygen radicals and certain nonradicals that are oxidizing agents. In physiological conditions, mitochondria are the major source of intracellular ROS. Hyperglycemic conditions increase electron flux through the respiratory chain in mitochondria stimulating the formation of ROS. The key enzyme for ROS production in cells is NADPH oxidase. NADPH oxidase is a multisubunit enzyme comprising membrane and cytosolic component, which responses to environmental and micronutrient stimulants. The NADPH oxidase-mediated release of ROS in macrophages, also called *respiratory burst* (sometimes called oxidative burst), leads to the elimination of invading microorganisms [3]. Under unstimulated conditions, the multidomain regulatory subunits, p40phox, p47phox, and p67phox, exist as a complex in the cytosol. Upon stimulation, p47phox undergoes phosphorylation, translocates to the membrane to activate NADPH oxidase and produces superoxide [4]. In human monocytes and murine macrophages PI3K and protein kinase C (PKC) pathways are involved in NADPH oxidase stimulation. This mechanism triggers ERK1/2, p38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB, which terminates in the activation of monocytes and proliferation of macrophages [5].

1.2. *Reactive nitrogen species*

Reactive nitrogen species oxidize proteins and nucleic acids. In addition to producing ROS, the mitochondrial respiratory chain is capable of producing nitric oxide (NO) [6]. Inducible NO synthase (iNOS) is a key enzyme in the macrophage that is potently induced in response to proinflammatory stimuli. Macrophages are activated by interferon-γ and microbial products such as lipopolysaccharides (LPS), leading to production of proinflammatory cytokines and high levels of NO. NO is a potent molecule involved in critical macrophages functions such as cytotoxicity against intracellular pathogens, viruses and tumors, and immune regulation [7].

1.3. *Reactive carbonyl species increase nitric oxide and superoxide generation*

An increase in steady-state level of reactive carbonyl species (RCS) may cause *carbonyl stress*. Methylglyoxal (MG) is one of the most studied RCS formed during glucose, protein and fatty acid metabolism and is increased especially in hyperglycemia conditions. An excess of MG formation can increase ROS formation and advanced glycation end products, and cause oxidative stress. RCS including MG and advanced glycation end products are also associated with the age-related diseases such as cardiovascular complications of diabetes, neurodegenerative diseases, and connective tissue disorders through increasing oxidative stress [8].

1.4. *Macrophages are the major sources of oxidative stress*

In macrophages NO is synthesized by iNOS; while superoxide is mainly produced by NADPH oxidase. The reaction of superoxide with NO leads to the formation of peroxynitrite *in vivo*. This iNOS-derived peroxynitrite results in nitrotyrosine formation, increased antibacterial activity, and cytotoxic actions of macrophages [9]. Recent evidence indicates that peroxynitrite contributes most of the cytotoxicity of resident macrophages. Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or indirect radical-mediated mechanisms. These reactions trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury. *In vivo*, peroxynitrite generation has been attributed to inflammatory diseases such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, cancer, and neurodegenerative disorders [10].

2. Epigenetic mechanism for disease pathogenesis

2.1. *Tissue-resident macrophages*

Tissue-resident macrophages are nonspecific killer cells that eliminate bacteria, foreign bodies, dead cells, and debris and recruit monocyte/macrophages in response to inflammatory signals. Specialized tissue-resident macrophages include osteoclasts (bone), alveolar macrophages (lung), histiocytes (interstitial connective tissue), Kupffer cells (liver), Langerhans (skin), microglia (brain), and mesangial cells (kidneys). Tissue-resident macrophages may initiate the inflammatory response depending on the nature of the insult and its magnitude [11].

2.2. *Macrophage activation and inflammation*

Macrophages are differentiated into phagocytes with immune and homeostatic functions. Macrophages can either be classically (M1) or alternatively (M2) activated dependent on the stimulus and the resulting phenotype of the cell. Classical activation (M1) of macrophages by LPS through Toll-like receptor 4 (TLR4) has been well characterized. An alternative