Antioxidant Effects and Mechanisms of Tea Polyphenols: Postprint

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Date: 2017-10-23T00:00:00+00:00

Abstract

Tea polyphenols (TP) are a natural compound extracted from green tea and black tea, possessing biological functions such as free radical scavenging, prevention of DNA damage, regulation of intracellular antioxidant defense systems, and anticancer activity. This article systematically reviews the composition of TP, its antioxidant effects, the regulation of the mitogen-activated protein kinase-nuclear factor erythroid 2-related factor 2-antioxidant response element (MAPK-Nrf2-ARE) signaling pathway, and the associated mechanisms.

Full Text

Anti-oxidation Functions of Tea Polyphenols and Its Mechanisms

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Abstract: Tea polyphenols (TP) are pure natural compounds extracted from green tea and black tea that can scavenge free radicals, protect DNA from damage, regulate intracellular antioxidant defense systems, and exhibit anti-cancer properties. This paper systematically reviews the composition of TP, their antioxidant functions, and their regulatory effects on the mitogen-activated protein kinase (MAPK)-nuclear factor erythroid-2-related factor 2 (Nrf2)-antioxidant response element (ARE) signal transduction pathway and related mechanisms.

Keywords: tea polyphenols; anti-oxidation functions; oxidative stress; MAPK-Nrf2-ARE signal transduction pathway

With the improvement of dairy farming standards and increasing stocking density in China, milk production has risen, which simultaneously intensifies metabolic processes and generates substantial free radicals in the body. The increased metabolic demands during the periparturient and peak lactation periods promote the production of reactive oxygen species (ROS) in dairy cows. Under normal conditions, ROS maintain a dynamic equilibrium that supports proper metabolism and immune function. However, when ROS generation exceeds the body' s scavenging capacity, lipid peroxidation occurs, leading to oxidative stress. This condition alters cell membrane structure and function, reducing production performance, inflammatory response capacity, and immune function, while increasing susceptibility to diseases such as mastitis, retained placenta, metritis, ketosis, abomasal displacement, and fatty liver. Therefore, enhancing animal antioxidant capacity has become an urgent issue in dairy production.

Currently, numerous natural antioxidants with similar functions are available, including vitamin E, β-carotene, vitamin C, curcumin, and tea polyphenols (TP), all of which protect cell membranes and scavenge intracellular free radicals to prevent various diseases and improve immunity. TP serves as an effective ROS scavenger and a strong reductant that undergoes redox reactions with biological systems to eliminate oxygen and lipid free radicals, thereby preventing lipid peroxidation, protecting DNA from damage, inhibiting tumorigenesis, and delaying aging. Research indicates that when animal bodies are stimulated by extracellular environmental factors, TP regulates transcription factor activity by inhibiting phosphorylation of signaling pathways dominated by activating protein 1 (AP-1) and nuclear factor-kappa B (NF- B). TP primarily modulates the MAPK-Nrf2-ARE signal transduction pathway to influence the expression of apoptosis-related genes and proteins. This review focuses on the antioxidant functions of TP and its regulatory mechanisms on the MAPK-Nrf2-ARE pathway, providing a theoretical basis for ensuring healthy dairy farming, alleviating oxidative stress damage, and preventing disease.

Oxidative stress is a pathological process resulting from an imbalance of oxygen free radicals that causes physiological changes and disease. Excessive ROS generation damages DNA molecules and induces cellular lipid peroxidation, leading to massive cell death. ROS also interacts with NF- B to regulate cellular signal transduction pathways. NF- B plays a central role in transcriptional regulation of cell information mediated by various stimuli and participates in the expression and regulation of multiple genes, serving as a marker of cell activation. NF- B dysfunction can cause various diseases, with mastitis being the most common in dairy cows. During lactation, vigorous metabolism makes dairy cows highly susceptible to oxidative stress, generating excessive oxygen free radicals and lipid peroxides. If these radicals cannot be promptly eliminated, they cause cell aging and apoptosis, leading to oxidative damage in mammary tissue and reduced antioxidant capacity, which compromises milk quality. Therefore, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), along with natural antioxidants like vitamin E, vitamin

C, and TP, are needed to regulate transcription factor activity and alleviate oxidative damage caused by ROS. These antioxidants play crucial roles in eliminating harmful free radicals and preventing inflammatory damage and disease development.

2. Composition and Structure of Tea Polyphenols

Tea polyphenols are the general term for phenols and their derivatives in tea, representing an active component comprising approximately 30% of the dry weight of tea products. The chemical composition mainly includes flavanols (catechins), anthocyanins, flavones and flavonols, and phenolic acids, with catechins being the most important components. The four primary catechins are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), among which EGCG exhibits the highest antioxidant activity, accounting for approximately 50% of total catechins. TP are flavonoid compounds based on the α-phenylbenzopyran structure, where hydroxyl substituents serve as proton donors, conferring special physiological functions including anti-aging, anti-radiation, anti-tumor, and antibacterial effects. These functions are closely related to their free radical scavenging capacity. [Figure 1: see original paper] illustrates the chemical structure of tea polyphenols.

The structural formulas are as follows: Epicatechin (EC): R1=H, R2=H; Epigallocatechin (EGC): R1=H, R2=OH; Epicatechin gallate (ECG): R1=X, R2=H; Epigallocatechin gallate (EGCG): R1=X, R2=OH.

Fig.1 The chemical structure of tea polyphenols[15]

3.1 Scavenging Oxygen Free Radicals

As a reductant with low redox potential, TP can reduce oxygen radicals to relatively stable compounds. The phenolic hydroxyl groups in TP provide hydrogen atoms and undergo oxidation to form stable radicals containing catechol structures, thereby scavenging harmful free radicals in the body. By eliminating ROS, TP significantly reduces malondialdehyde (MDA) content and lactate dehydrogenase (LDH) activity in mammary epithelial cells while increasing SOD activity, thereby alleviating apoptosis, reducing lipid peroxidation, and protecting dairy cow mammary epithelial cells from ROS-mediated damage to cell membrane structure and function. Additionally, TP exhibits similar characteristics to vitamin E in terms of inhibition time, proliferation rate, kinetic chain length, and stoichiometric coefficient, and demonstrates synergistic effects with vitamin E that enhance free radical scavenging capacity and improve the body' s antioxidant level.

3.2 Regulating Intracellular Antioxidant Defense Systems

The intracellular antioxidant defense system directly scavenges free radicals to maintain their dynamic equilibrium. TP synergistically enhances the defensive

functions of radical-related substances including SOD, CAT, GSH-Px, vitamin E, vitamin C, and glutathione (GSH), while inhibiting oxidases such as cyclooxygenase (COX), lipoxygenase (LOX), and xanthine oxidase (XOG). TP significantly upregulates mRNA expression levels and protein synthesis of SOD, CAT, and GSH-Px in cells, increasing their activity in culture medium to effectively eliminate free radicals and reduce mitochondrial damage caused by oxidative stress. TP also regulates the antioxidant defense system by modulating transcription factor gene expression. Under normal physiological conditions, TP does not significantly affect NF- B, Jun, and Fos expression; however, during oxidative stress, when these factors are upregulated, TP significantly downregulates their expression. Since AP-1 activity is regulated by inhibiting Jun and Fos expression, TP can protect animals from oxidative stress damage through modulation of NF- B and AP-1 signaling pathways.

3.3 Protective Effect on DNA Damage

TP indirectly eliminates excessive oxygen free radicals by enhancing antioxidant enzyme activity and altering protein structure and function. During oxidative stress, excessive ROS primarily attacks cellular DNA, inducing gene mutations and carcinogenesis. Copper ions (Cu^{2+}) and hydrogen peroxide can induce DNA damage, but TP can effectively reduce DNA single-strand breaks by directly competing for damage energy or interacting with damage products, thereby exerting protective effects on DNA damage through interaction with target cell molecules within a certain timeframe.

3.4 Anti-cancer Effects

Tumor formation is a multi-factorial, multi-step, multi-gene mutation process. TP exerts anti-cancer effects through multiple mechanisms, including free radical scavenging, inhibition of metabolic transformation, suppression of pro-carcinogenic enzyme metabolism, alteration of mitochondrial permeability, induction of tumor cell DNA double-strand breaks, and reversal of multidrug resistance (MDR) in tumor cells. Additionally, TP promotes tumor cell apoptosis by activating the intrinsic mitochondrial pathway, where caspase-3 plays a crucial role. Normally present in the cytoplasm as a proenzyme, caspase-3 is activated during early apoptosis to cleave corresponding cytoplasmic and nuclear substrates, leading to cell death and thereby exerting anti-cancer effects.

4 Regulatory Mechanisms of Tea Polyphenols on Intracellular Signal Transduction Pathways

Animals generally defend against oxidative stress damage by regulating their endogenous antioxidant defense systems to maintain the balance between ROS generation and elimination. Extracellular stimuli transmit signals through various cellular signaling pathways, and the body' s responses to stimuli are also

mediated through these transduction pathways. Therefore, antioxidant activity is not the sole mechanism by which TP protects cells. TP also effectively promotes cell growth and protects cells from oxidative damage by influencing intracellular signal transduction pathways including MAPK, protein kinase C (PKC), and phosphatidylinositol 3-kinase (PI3K).

The MAPK-Nrf2-ARE pathway is a crucial defensive signal transduction pathway that regulates redox status and mediates TP' s regulatory mechanisms in response to external stimuli. Nrf2 activation is a prerequisite for initiating the Nrf2-ARE signaling pathway. Under normal cellular conditions, Nrf2 remains in a relatively inhibited state by binding to cytosolic Kelch-like ECH-associated protein-1 (Keap1). Upon external stimulation, the two cysteine sites c273 and c288 on Keap1 are simultaneously modified, causing Nrf2 to dissociate from Keap1, translocate into the nucleus, and bind to ARE to initiate downstream gene transcription, thereby expressing TP-regulated genes and alleviating oxidative stress damage.

Phosphorylation is the primary mechanism through which Nrf2 exerts its antioxidant stress effects. Under normal physiological conditions, Nrf2 bound to Keap1 becomes a target for protein kinases and undergoes degradation. During oxidative stress, Nrf2 phosphorylation alters its conformation, reducing recognition by protein kinases, increasing Nrf2 content and stability, and promoting its nuclear translocation to initiate downstream gene transcription. The MAPK signaling pathway also regulates Nrf2-ARE pathway activation and dependent gene expression. TP modulates the dynamic balance of the body' s antioxidant defense system by regulating four major MAPK signaling pathways: extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinases (JNK), p38 mitogen-activated protein kinases (p38MAPK), and extracellular signal-regulated kinases 5 (ERK5). TP primarily regulates p38MAPK and ERK1/2 signaling pathway activity by promoting Nrf2-mediated heme oxygenase-1 (HO-1) expression, reducing ROS generation and oxidative stress-induced cell damage, thereby decreasing disease incidence caused by oxidative stress.

In summary, the MAPK-Nrf2-ARE signal transduction system is an important regulatory pathway for TP' s effects on intracellular physiological and biochemical reactions, playing vital roles in various organisms. This pathway contains corresponding substrates and protein kinases that can receive external and internal signals to elicit different cellular responses, weaving these signals into a complex network. Intracellular signal transduction is a sophisticated network system where different signaling pathways exhibit universality, independence, and specificity. TP determines the antioxidant effects exhibited by cells after oxidative stress through interactions (synergistic or antagonistic) among various signaling pathways.

TP can alleviate oxidative stress damage by regulating antioxidant enzyme activity and protein kinase-related gene expression, demonstrating significant effects in improving antioxidant and immune functions. However, current research has primarily focused on human medicine, with limited reports on TP' s effects on

oxidative stress-induced damage in ruminants and related antioxidant mechanisms. Therefore, systematically investigating TP' s regulatory effects on the KEAP1-Nrf2-ARE signaling pathway at the gene and protein expression levels, and studying TP' s antioxidant functions in dairy cow mammary tissue, will provide theoretical foundations for scientifically supplementing TP in dairy production to ensure mammary health and produce high-quality milk.

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