

Multifaceted Anti-Aging Mechanisms of Plant-Derived Bioactive Peptides: Immunomodulation, Mitochondrial Metabolism, Autophagy/Apoptosis, and Stem Cell Regeneration

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Abstract. Population aging is closely connected with the process of decline of physiological function and to the increase of the prevalence of age-related diseases. Plant-derived bioactive peptides (PBPs) have been a subject of much interest in recent years as natural bioactive compounds that may serve as natural health promoters and anti-aging agents. The peptide substances possess great biocompatibility, low toxicity and several biological activities. This article will discuss the possible anti-aging action of PBPs in the light of recent experimental results. PBPs have the power to limit oxidative damage by eliminating reactive oxygen and strengthening the natural antioxidant defense system. In addition, they can also regulate the immune response and inhibit chronic low-degree inflammation by regulating cytokine production and maintaining immune homeostasis. Plant peptides (PBPs) stimulate mitochondrial function and energy metabolism by triggering essential energy metabolism signaling pathways involved in the biosynthesis of mitochondria. Besides, some peptides of plant origin have been shown to inhibit the excessive apoptosis and promote autophagy, contributing to the quality control of cells and integrity of tissues. Recent research studies also indicate that PBPs can promote the growth of stem cells and regeneration of tissues by controlling growth associated signaling pathways. To conclude, current evidence suggests that plant-derived bioactive peptides could target several aging-related targets simultaneously and is a promising candidate drug in the field of healthy aging intervention. Further studies are still required which should include bioavailability, molecular mechanism and clinical verification to support its future use in functional food and anti-aging therapy.

Keywords: Plant-derived bioactive peptides, Anti-aging, Oxidative stress, Mitochondrial function, Autophagy

1. Introduction

Population aging is one of the most critical public health concerns in the world today in the 21st century. Age-related diseases such as cardiovascular diseases, neurodegenerative diseases, metabolic syndrome and degeneration of tissues have sharply increased with the increased life span. Aging is now known to be a multi-factors biological process, that involves the progressive decline of cells and physiological functions. Physiological decline and age-related diseases are caused by a variety of core aging characteristics, primarily oxidative stress, chronic inflammation, mitochondrial dysfunction, apoptosis disorder, autophagy damage and stem cell depletion. Oxidative stress is one of the main factors leading to aging. A surplus of reactive oxygen (ROS) will lead to the collapse of cell homeostasis and help the progression of aging-related diseases. In recent years, plant-based bioactive peptides (PBPs) have attracted much attention from the scientific community because of their strong antioxidant activity and good safety. These peptides typically are liberated from plant proteins by enzymatic hydrolysis, fermentation or food processing, and tend to be made up of short amino acid sequences of high biological activity.

Practical use of PBPs is characterized by high bioavailability and low toxicity, and the PBPs have excellent biocompatibility unlike the synthetic antioxidants. Many experimental studies have already demonstrated that peptides derived from plants are effective in scavenging free radicals, boosting the activity of the body's own antioxidant system, decreasing lipid peroxidation, and preventing damage to cell components by oxidation. Further structure analyses confirm that antioxidant activity of these peptides is closely related with the molecular weight, the percentage of hydrophobic amino acids and the frequency of aromatic residues, thus supporting their role in neutralisation of electrons and free radicals [1].

New evidence suggests that PBPs can have effects on cell growth, cell survival and tissue regeneration, in addition to their antioxidant properties. Corn protein derived peptides from Corn Alcohol Soluble Protein (CASP) have been shown to significantly stimulate proliferation of C2C12 myoblasts by synergistically stimulating the mTORC1 and mTORC2 signalling pathways. Protein synthesis, cell growth, metabolism and survival are regulated by these pathways and play an important role in maintaining tissue integrity during aging. The results of these studies provide strong evidence that the peptides derived from plants have an anti-aging effect, acting in two ways: by decreasing oxidative damage and by controlling tissue regeneration. Firstly, there is no one single molecular pathway to aging. In contrast, aging is not a simple event and involves multiple factors including oxidative stress, immune dysfunction, decreased mitochondrial activity, increased levels of cell apoptosis and loss of stem cells. Current research has demonstrated that plant-based bioactive peptides (PBPs) are able to act on several aging associated signaling pathways simultaneously, which results in synergic protective effects on the physiological functions. Therefore, plant-derived bioactive peptides are natural candidates for use as drugs with a high potential in healthy aging intervention and functional food development [1-3].

The present study aims to putatively summarize the anti-aging mechanism of plant derived bioactive peptides centering on its inflammatory and immune regulatory mechanism, mitochondrial function and energy metabolism, apoptosis and autophagy, and stem cell mediated tissue regeneration (Table 1). A complete understanding of these molecular mechanisms can lead to feasible theoretical developments of the next generation of nutritional health care products and anti-aging therapies that use peptides.

2. Anti-inflammatory activity and immune regulation

Since low degree inflammation is present in some individuals for a long time, it has been suggested that it constitutes a marker of biological aging, sometimes referred to as "inflammatory aging". Inflammatory aging is not a protective response to resistance to microorganisms and damage to tissues as is the case in acute inflammation, but involves the gradual accumulation of pro-inflammatory mediators in the body. Numerous clinical and preclinical studies have demonstrated that chronic inflammation is a major pathogenic trait associated with many chronic diseases, such as cardiovascular diseases, type 2 diabetes, neurodegenerative diseases and cancer, all of which are related to the aging process. Hence, modulation of the inflammatory response and immune-homeostasis is essential to slow down aging and extend healthy life. The immune system will go through significant structural and functional changes, called immune aging. The changes are reduction of lymphocyte proliferation, diminished activity of the macrophages, disorder of the production of cytokines, and reduced adaptive immune response. With the decline of immunity, the body's susceptibility to infection, chronic inflammation and tissue damage also increases. Thus, interventions with the potential to restore immune balance can have a substantial impact in slowing down the aging process. Recently, bioactive peptides from plants are emerging as highly promising as immunomodulators.

Table 1. Summary of anti-aging mechanisms and regulatory pathways of plant-derived bioactive peptides

Peptide source	Regulatory pathways/targets	Main biological effects	Ref.
Zein-derived peptides (e.g., FLPFNQL)	Inhibit the production of pro-inflammatory cytokines (IL-6, TNF- α)	Reduce chronic low-grade inflammation; maintain the stability of the immune environment	[4]
Soybean peptide (VH-4-A)	Activation of AMPK α 1/Sirt1/PGC1 α /FoxO3a cascade	Promotion of mitochondrial biogenesis; improvement of respiratory chain efficiency; enhancement of cell survival under stress	[5]
Walnut small-molecule oligopeptides	Upregulation of mitochondrial biogenesis-related enzymes; enhancement of hepatic and muscular glycogen stores	Prolonged swimming time; reduced blood lactate and blood urea nitrogen; alleviation of exercise-induced fatigue	[6]
Rapeseed peptide (WDHHAPQLR)	Inhibit the activation of Caspase-3/9; increase the Bcl-2/Bax ratio; maintain the mitochondrial membrane potential	Suppression of H ₂ O ₂ -induced apoptosis in vascular endothelial cells	[7]
Broccoli stem protein hydrolysates	Upregulation of structural and metabolic proteins involved in epidermal remodeling	Promotion of HaCaT keratinocyte proliferation; acceleration of wound healing (within 24–48 h)	[8]
Soy peptide	Activation of ERK (p44/42 MAPK) pathway and downstream mTOR/p70S6K/eIF4E;	Promote the proliferation of adipogenic and umbilical mesenchymal stem cells; maintain the self-renewal ability of stem cells	[9]

This review explores extensively the immunomodulatory peptides produced by plants such as soybeans, rice, wheat, oats, corn and others, highlighting their ability to modulate innate and adaptive immune responses. Such peptides, which are generally generated by enzyme hydrolysis, are biologically active in relation to the composition and structural properties of the amino acids. As

opposed to the traditional anti-inflammatory drugs which will primarily target a single signaling pathway, the plant-derived peptides could target multiple components of the immune system simultaneously, which is more appropriate for employing them as a dietary anti-aging intervention. Until now, several mechanisms have been suggested for plant-derived peptides to act as immune regulators.

For one, these peptides can help to control the activity of macrophages, a primary defence mechanism of innate immunity. Activated macrophages will secrete cytokines and inflammatory mediators, hence coordinating the immune response. But if they are overactive, they can cause chronic inflammation and damage to the tissue. It has been demonstrated that immunomodulatory peptides are able to regulate the production of inflammatory cytokines, preventing excessive activation of the macrophages. Secondly, plant peptides can influence the proliferation and differentiation of lymphocytes and so preserve the adaptive immune function in the aging process. Third, they can control the levels of cytokines, including interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) which are thought to act as mediators between inflammation and aging [3]. Experimental evidence on these effects of corn-derived peptides has been reported. Liu et al. discovered several immunomodulatory peptides derived from corn alcohol soluble protein hydrolysate and examined their activity by using human macrophage-like U937 cell [4]. The peptides identified include FLPFNQL with the highest immunomodulatory activity that may decrease the production of IL-6 to approximately 58%. IL-6 is a major pro-inflammatory cytokine, the levels of which are frequently increased in the elderly and strongly linked with chronic inflammatory diseases. The inhibitory effect of corn alcohol soluble protein-derived peptides on the production of IL-6 suggests that these peptides have the potential to effectively inhibit age-related inflammatory response and contribute to the maintenance of immunostasis.

Note that oxidative stress and inflammation are closely related processes in the aging process. Over production of ROS will lead to activation of the inflammatory signaling pathway and chronic inflammation will further exacerbate the oxidative damage, create a positive feedback loop and accelerate the aging of the cells. As many plant-originated peptides are antioxidant and also have immunomodulatory property, they can break this vicious circle by double regulation. PBPs can overcome age-related deterioration of the body's functions as they have the potential to decrease oxidative stress, prevent overproduction of cytokines and balance immune responses.

To conclude, there are available evidences indicating that bioactive peptides derived from plants can be used as natural anti-aging agents capable of modulation of inflammatory and immune response. They help in regulating the generation of cytokines, the activity of macrophages, and adaptive immune response, which may be crucial in preventing inflammation and aging and in maintaining physiological homeostasis in the aging process.

3. Mitochondrial function regulation and energy metabolism

Biological aging is characterized by the main sign of mitochondrial dysfunction, which results in a reduction of ATP production and an increase of cell production of reactive oxygen (ROS). As the mitochondria get old, their inability to synthesize and work efficiently will speed up the process of aging and fatigue of the tissues. Recent studies have demonstrated that plant derived bioactive peptides (PBPs) have the capacity to be a good intervention for restoring the mitochondrial homeostasis and regulating the system energy metabolism [2]. But an increasing body of evidence indicates that the combination of particular soybean peptides and physiological intervention will be able to noticeably remove age-related metabolic decay. Among them, bioactive peptide VH-4-A from soybeans is demonstrated to combat the hypertension and the metabolic abnormality in anti-

aging rats [5]. Mechanism studies demonstrate that VH-4-A can effectively activate important metabolic longevity pathways, such as adenosine-activated protein kinase $\alpha 1$ (AMPK $\alpha 1$), Sirtuin 1 (Sirt1) and peroxisome proliferator activation, particularly when combined with exercise training. Receptor γ co-activator 1- α (PGC1 α) [5]. AMPK $\alpha 1$ /Sirt1/PGC1 α signal cascade activates downstream mitochondrial biosynthesis, optimizes the efficiency of the respiratory chain and upgrades Forkhead box O3 (FoxO3a), which promotes the survival of cells under metabolic stress [5].

In line with the above, the small molecule oligopeptides isolated from Walnut (*Juglans regia* L.) demonstrate considerable anti-fatigue and energy regulating properties in the bodies [6]. Animal behavior tests and biochemical tests showed that walnut oligopeptides could significantly prolong the weight-bearing swimming time of mice, prevent blood lactic acid and urea nitrogen from accumulating [6]. These peptides derived from walnuts can improve glycogenesis in liver and muscle tissue and the antioxidant systems in the mitochondria at the molecular level [6]. These PBP can both upregulate the expression of mitochondrial biosynthetic transduction protein and lower oxidative damage, which will both lead to an improvement in the structural degradation of the mitochondria [6]. To sum up, these data highlight the importance of PBPs in slowing down the physiological aging process through targeting interrelated mitochondrial signaling networks, which maintain metabolic plasticity and cellular energy capacity. This is a potential action of a compound that can inhibit cell death and stimulate the recycling of damaged cells.

4. Anti-apoptosis and autophagy activation

Programmed cell death pathways (primarily apoptosis and macroautophagy) are critical to maintain cell homeostasis in the aging process. The damaged macromolecules and functionally abnormal organelles accumulate with the aging of the organisms controlled by gradual imbalance of the cell quality control mechanism. This is reflected in both an increase and an imbalance of apoptosis in functional tissues and damage to autophagy, the predominant degradation pathway that relies on the lysosomes and is responsible for eliminating cytotoxic aggregates. The bioactive peptides produced from food proteins have emerged as critical regulators and are able to rebalance the programmed cell death network and therefore fight against inflammatory diseases and age-related degeneration of tissues [10]. Pathological aging and chronic stress can lead to a selective PBPs intervention on the apoptosis execution pathway [10]. For instance, a new peptide, named WDHHPQLR, from the rapeseed protein has a strong protective effect on oxidative stress induced cell death [7]. WDHHPQLR treatment can significantly inhibit cell apoptosis in hydrogen peroxide (H₂O₂) induced human umbilical vein endothelial cells (HUVECs) and is dose-dependent [7]. The mechanism of this anti-apoptosis effect involves inhibition of the activation of Caspase-3 and Caspase-9, keeping the mitochondrial membrane potential and down-regulation the expression of the apoptotic protein Bax and maintaining the anti-apoptotic protein Bcl-2 [7]. Meanwhile, PBPs in the diet may have anti-aging therapeutic effects by the recovery of autophagy flux [10]. During the aging process, autophagy inhibition will help to hold on to damaged mitochondria, thereby further augmenting the amount of intracellular reactive oxygen (ROS). Bioactive peptides are able to break this vicious cycle through activation of the upstream regulator of autophagy or the regulation of the mTOR/AMPK axis to stimulate phagocytosis and degradation of damaged cell components [10]. These peptides derived from plants may work synergistically to inhibit the premature apoptosis of key vascular cells and somatic cells, and induce autophagy clearance to maintain the quality control of cells and effectively delay the aging of cells and related tissue failure.

5. Stem cell function promotion and tissue regeneration

As the cells age, loss of stem cells at the level of tissues is also an important mechanism of tissue degeneration and the inability to regenerate. Over time the proliferation potential and differentiation efficiency of the stem cells, such as MSCs as well as the groups of progenitor cells in the local area will decline, primarily due to the pressure from the environment and the use of the endogenous growth factors. Thus, the main objective of anti-aging medicine is to discover natural drugs which will be able to maintain the self-renewal of stem cells while promoting regulated tissue regeneration. Recent breakthrough studies have revealed that biologically active peptides from plants have a dramatic impact on the re-vitalization of stem cells and progenitor cells [8]. Peptide hydrolysate from the total protein and membrane parts from broccoli stems has been demonstrated to improve keratinocytes' regeneration ability greatly [8]. These broccoli derived peptides are able to induce controlled proliferation of human HaCaT keratin-forming cells and in the 24-48 hours, they can act significantly in wound healing without inducing cytotoxicity [8]. Proteomics and gene expression spectrum analysis reveal that these peptides are able to upregulate the expression of the structural proteins and metabolic proteins involved in the remodeling of the epidermis and consequently strengthen the outer barrier of the epithelia that typically weakens due to aging [8]. Besides, in serum-free microenvironment, plant-based soybean peptide was demonstrated to be an active constituent to maintain the microenvironment of adult stem cells (ASC) [9]. Experimental data has demonstrated that peptides from the soybeans are able to substantially stimulate the proliferation of mesenchymal stem cells from human adipose tissue (ADSCs) and mesenchymal stem cells from umbilical cord blood (CB-MSCs) [9]. Mechanistically, soybean peptides are able to sequentially activate the p44/42 mitogen-activated protein kinase (ERK) pathway and downstream translation control factors, such as the mechanistic target of rapamycin (mTOR), p70 S6 kinase and eukaryotic initiator 4E (eIF4E) in ADSCs [9]. Simultaneously, these peptides are able to quantitatively stimulate the autosecretion of endogenous peptides (transforming growth factor- β 1 (TGF- β 1), vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6)), that maintain the self-renewal of stem cells [9]. PBP are able to awaken stem cells from environmental dormancy by co-activation of both ERK and TGF- β 1 pathways and therefore offer a feasible treatment for aging related tissue engineering and systemic regeneration. The bioactive peptides derived from plant are in an increasing trend and are emerging as a highly potential natural compound for anti-aging due to their diversified biological activities and good safety. The current evidence indicates that plant-based peptides (PBPs) may influence various basic aging mechanisms, such as oxidative stress, chronic inflammation, mitochondrial dysfunction, disorder of cell apoptosis and stem cell exhaustion. These peptides influence various signaling pathways, which aids in maintaining cellular homeostasis, boosting energy metabolism, enhancing immune balance, promoting tissue repair and regeneration. Importantly, the anti-aging effect of PBPs appears to be by regulating a set of interrelated biological systems, and not by regulating any one target. This multi-target feature could make it superior to traditional interventions to target a single aging pathway. However, currently most findings are from cell or animal studies and evidence from clinical trials in humans is still scarce. Moreover, there are issues of stability, absorption efficiency and large-scale application of peptides that need to be addressed. Further studies need to be done to elucidate the relationship between peptide binding proteins (PBP), enhance its bioavailability and confirm its effectiveness in well-designed clinical studies. A thorough understanding of these processes can aid in the creation of functional foods and nutritional health products that help to prevent age-related diseases and promote healthy aging using peptides.

6. Conclusion

Current studies have demonstrated that plant-derived bioactive peptides (PBPs) have achieved multi-dimensional breakthroughs in the field of anti-aging, with their effects covering core processes including inflammatory immunity, mitochondrial metabolism, cellular apoptosis and autophagy, and stem cell regeneration. In terms of anti-inflammatory immunity, such peptides can regulate the activity of macrophages and lymphocytes, modulate the secretion of inflammatory factors, break the vicious cycle between oxidative stress and chronic inflammation, effectively alleviate inflammation and restore immune homeostasis. For the regulation of mitochondrial function, specific peptides can activate key signaling pathways such as AMPK/Sirt1/PGC1 α , facilitate mitochondrial biogenesis and optimize the efficiency of the respiratory chain, boost cellular energy metabolism, and counteract age-related physical decline. In response to disorders of cellular apoptosis and autophagy, PBPs suppress excessive programmed cell death and restore autophagic flux, eliminate toxic proteins and damaged organelles, and maintain the cellular quality control network. With regard to stem cell regeneration, these peptides activate the ERK pathway and its downstream mTOR pathway, stimulate the secretion of endogenous growth factors, promote the proliferation and differentiation of mesenchymal stem cells, and accelerate tissue repair and regeneration.

Overall, the superiority of PBPs lies in multi-target synergistic regulation rather than single-pathway intervention, offering a natural strategy for delaying multi-system aging. Looking ahead to future development, efforts should focus on advancing clinical translational research to verify their long-term efficacy and safety; improving peptide stability and bioavailability via technological innovations such as structural modification and nano-delivery; utilizing systems biology and omics technologies to thoroughly elucidate their regulatory networks; exploring synergistic effects when combined with interventions including exercise and dietary restriction; and developing green and efficient large-scale preparation processes to meet industrialization demands. The systematic advancement of these research directions will help PBPs evolve into functional foods or adjuvant therapeutic agents for promoting healthy aging.

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