Review Article

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Proanthocyanidins: A Nutraceutical Host Modulatory Weapon Against Chronic Periodontitis

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ABSTRACT

Background: Polyphenolic compounds of plant origin have the potential for improving human health. Flavonoids are the most common group of polyphenolic compounds. Proanthocyanidins are oligomers and polymers of monomeric flavonoids found in different fruit products including apple juice, purple grape juice, green tea, cranberries and dark chocolate. They are beneficial molecules in preventing or modifying the pathophysiology of many diseases and chronic inflammatory conditions like periodontitis. This review describes the different beneficial roles that proanthocyanidins can offer as a nutraceutical host modulatory weapon against chronic periodontitis.

Keywords: Polyphenolic compounds, Flavonoids, Proanthocyanidins, Periodontitis.

INTRODUCTION

Polyphenolic compounds are common in all plants. While polyphenolic compounds do vary from species to species of different plants, their potential for improving human health, when they are consumed, is the same.[1,2] Flavonoids are the most common group of polyphenolic compounds. They are found ubiquitously in plants and they occur naturally in foods derived from plants. Flavanoid containing formulations have been used in folk medicine among several races for obtaining preventive and therapeutic benefits. Foods with a high flavonoid content include parsley, onions, blueberries and other berries, black tea, green tea, oolong tea, bananas, all citrus fruits, pomegranate, red wine, sea-buckthorns, dark chocolate (with a cocoa content of 70% or greater) etc. Flavonoids have potential clinical effects including anti-inflammatory, antitumor, antiatherosclerotic, antithrombogenic, antiosteoporotic, and antiviral properties.[3]

Chemically, flavanoids are generally a 15-carbon skeleton, that consists of two phenyl rings (A and B) and heterocyclic ring (C). Proanthocyanidins (condensed tannins, or oligoflavanoids) are oligomers and polymers of monomeric flavonoids. Proanthocyanidins (PACs) more specifically are polyflavans: condensed molecules of those flavonoids with a saturated C- ring. PACs in sizes ranging from dimers through very large polymers are found in many plants. PACs are mainly concentrated in tree barks and outer skins of seeds. PACs are secondary metabolites of plants; that is, they are not required for the structural or metabolic integrity of the organism. The main function of PACs within the plant is to protect against microbial pathogens and pests.[4] Fifteen subclasses of PACs have been identified; however, only a few are prominent in foods and supplements of plant origin.[5] PACs consist of monomer flavan-3-ol units. When linked through either C4 to C8 or C4 to C6 bonds, the linkages are called B-type. When the linkages were through C2 and C7 compound, they are called A-type.

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There are simple and complex forms of A type and B linked PACs.[4] While B-linked PACs can be found in different fruit products including apple juice, grape juice, green tea and dark chocolate, A-linked PACs are found mainly in cranberries.

The unique polyhydroxy phenolic nature of PACs have given them substantial antioxidant potential and radical scavenging activity usually 20 times greater than Vitamin C and 50 times more potent than Vitamin E, the gold standards.[6] Due to the potent antioxidant activity, PACs have been the subject of recent research, making them a therapeutic weapon for the treatment of several conditions.

Usually, the initial response when a food or supplement containing high levels of PAC is consumed is an astringent sensation. As a result, palatable formulations of foods and supplements containing substantial levels of PACs have been a challenge for food formulation experts. Early studies employing extracts of "whole" grapes suggested PACs were absorbed and metabolized in rats and mice.[7] Later experiments using "purified" PAC fractions or grape seed extracts failed to demonstrate absorption of these food components in rats.[8] Studies with human subjects have corroborated these results, showing that dimeric proanthocyanidins, the higher polymers were not identified in plasma after consumption of flavonol-rich cocoa (containing primarily large polymers) or PAC-rich grape seed extract.[9,10]

Grape seed extract is being studied intensively as a source of PACs in view of its health-promoting potential in a number of model cell and animal systems. There are many different brands of grape seed extracts available in countries such as the United States, Australia, Japan and Korea. One of the mostly prevalent products is a novel IH636 grape seed proanthocyanidin extract available as "ActiVin" from Inter Health Neutraceuticals Incorporated. It is composed of a water-ethanol extract of red grape seeds. The daily recommended dosage is 50 mg for adults ages 30 years to 40 years, 100 mg for adults ages 40 years to 50 years, and 200 mg for adults older than 50 years. A dosage of 1 mg/kg/body weight has also been suggested.

Studies have shown evidence that the A-linked PACs from cranberries do have bacterial anti-adhesion properties.[11,12] PACs from grapes, apple juice, green tea and dark chocolate were tested against A-linked PACs of cranberry. The antiadhesion activity was observed after cranberry juice consumption but not in the other food which contain PACs.[11] The anti-adhesion properties gained by consuming cranberry powder was dose dependent and was prolonged with higher amounts of PAC equivalents.

The biological properties of PACs are being investigated as "natural" sources of drugs as "nutraceuticals". There are no known interactions between PACs extracts and other medications; however, data indicate PACs have an inhibitory effect on platelet aggregation similar to aspirin.[13] Therefore, caution is suggested in patients taking anticoagulant medication.[14,15] PACs have an excellent safety profile, with no known side effects, toxicity, or drug interactions. Rat studies have demonstrated PACs to be nonmutagenic and nontoxic at high levels. Human safety and toxicity studies for PACs are limited, but no side effects are reported in the literature. This review describes the different beneficial roles that proanthocyanidins can offer as nutraceutical host modulatory weapon against chronic periodontitis.

Mechanisms of action of PACs

1. Antioxidant property:

Oxidation is a natural process in the body where oxygen combines with reduced carbon based molecules (carbohydrates or fats) to provide energy. Cells cannot function efficiently when there is decreased oxidation or decreased energy production and disease results. "Oxidative stress" is created whenever there is an imbalance in this oxidant-antioxidant ratio. Antioxidants prevent these free radicals from causing excess cellular damage.[16]Chemically, the important features of flavonoids, are their remarkable antioxidant properties. The presence of electron-donating groups attached to the aromatic ring such as –CH3 and –OH increases the ease of hydrogen atom abstraction. The hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids, enable them to undergo redox reactions scavenging free radicals more easily.

PAC has been shown as antioxidants through the following mechanisms viz., (i) free radicals scavenging property and (ii) metal chelating activity. The protective role of PAC through its free radical scavenging property has been demonstrated by Ye et al. who related the scavenging capacity to the greater number of hydroxyl groups on the flavonoid nucleus.[17] The dimeric PACs are more effective than vitamin C in trapping oxygen radicals. PAC exerts inhibitory effect on the ROS generation as well as the lysosomal enzymes release. PAC easily releases electrons to free radical species like superoxide anion, hydroxyl, peroxyl and nitric oxide radicals. By release of electrons, the radical character of the ROS is transferred to PAC.

2. Antiinflammatory effects:

Certain phenolic compounds have been shown to inhibit the COX and 5-LOX pathways and thereby reducing the release of arachidonic acid. PAC could efficiently limit the inflammatory response of activated neutrophils in vitro. Altered expression of cell adhesion molecules has been observed in a variety of chronic inflammatory conditions. Albrecht et al[18] evaluated the effects of grape seed proanthocyanidin extract (GSPE) on the expressions of TNFα-induced ICAM-1 and VCAM-1 in primary human umbilical vein endothelial cells (HUVEC). The authors found that GSPE at low concentrations (1-5 µg/ml) could down-regulate TNFα-induced VCAM-1 expression. The same was not seen in ICAM-1 expression. The mRNA expression levels also exhibited such regulation of inducible VCAM-1 by GSPE.

A cell-cell co-culture assay was performed to verify whether the inhibitory effect of GSPE on the expression of VCAM-1 was effective in down-regulating actual endothelial cell/leukocyte interaction. GSPE treatment significantly decreased TNFα-induced adherence of T-cells to HUVEC. Although several studies have postulated NF-κB as the molecular site where redox active substances act to regulate agonist-induced ICAM-1 and VCAM-1 gene expression, inhibition of inducible VCAM-1 gene expression by GSPE was not through a NF-κB-dependent pathway as detected by a NF-κB reporter assay. The potent inhibitory effect of low concentrations of GSPE on agonist-induced VCAM-1 expression suggests therapeutic potential of this extract in inflammatory conditions and other pathologies involving altered expression of VCAM-1.

3. Nitric oxide synthase (NOS) activity:

While a small amount of NO is essential to maintain normal body homeostasis, a significant increase of NO activates inflammatory process. Flavanoids can repress NO production in macrophages and human peripheral blood mononuclear cells. Xia et al.[19] have shown that PAC from grape seeds decreases the levels of ROS, the NOS activity and the content of NO in rats.

4. Antimicrobial activity:

The antimicrobial effects of several tannin extracts on yeast, filamentous fungi, bacterial and viral toxicity have been reviewed by Chung et al.[20,21] Polymeric PAC as suppressors of antibiotic resistance in Staphylococcus aureus have shown promise as an alternative treatment to antibiotic use against Staphylococcus aureus infection.

Role of PACs in periodontitis

Periodontitis is a chronic inflammatory disease, characterized by an inflammatory host response against microorganisms of the bacterial plaque and their products. Periodontitis destroys the connective tissue and bone that support teeth. Bacteria like *Aggregatibacter actinomycetemcomitans* , *Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannarella forsythus* etc are implicated in the pathology of periodontitis. Bacteria induce tissue destruction indirectly by activating host defense cells, which in turn produce and release mediators that stimulate the connective tissue breakdown. Mediators like proteinases, cytokines and prostaglandins are produced as a part of the host response that contributes to tissue destruction. The periodontopathic bacteria trigger neutrophils to release ROS, which includes hydroxyl radical, superoxide anion and hydrogen peroxide. Increased cellular production of ROS is linked to the destruction of periodontium during chronic stages. Bacterial endotoxin especially lipopolysaccharide (LPS), activates macrophages to synthesize and secrete molecules including IL-1 and TNF-α, prostaglandins E2 (PGE2) and hydrolytic enzymes. These cytokines manifest potent proinflammatory

and catabolic activities resulting in periodontal tissue breakdown.

In recent years, there has been a tremendous stress on free radicals and antioxidant defense mechanisms. The human system contains a number of protective antioxidant mechanisms. Antioxidants either block the initiation of free radical formation or scavenge free radicals and terminate radical damage. Public awareness of the antioxidant and free radical-scavenging abilities of PAC compounds results largely from the potential of these compounds to increase human health in many ways. Several studies have been conducted in the recent past to assess the protective role of PAC in periodontitis. These include experiments in vitro and in vivo with type A linked and type B linked PAC. Since oxidative stress is encountered in periodontitis, the effect of PACs on periodontal inflammation is reviewed in this article.

In a study to assess the protective effects of grape seed proanthocyanidins against oxidative stress induced by lipopolysaccharides of periodontopathogens, Houde et al[22] treated macrophages with non-toxic concentrations of grape seed proanthocyanidin extract (GSPE) and compared with the effects produced by commercially available polyphenols like gallic acid [GA] and [−]epigallocatechin-3-gallate [EGCG]. They were then stimulated with lipopolysaccharide (LPS) of *Aggregatibacter actinomycetemcomitans* or *Fusobacterium nucleatum*, and iNOS expression was evaluated by immunoblotting. Nitric oxide (NO) and ROS production were quantified. GSE strongly decreased NO and ROS production and iNOS expression by LPS-stimulated macrophages. GA also revealed a strong inhibitory effect on NO production without affecting iNOS expression but slightly increasing ROS production. EGCG showed an inhibitory effect on NO and ROS production and on iNOS expression by macrophages.

Bodet et al[23] investigated the effect of a PAC-enriched cranberry fraction(C-PAC), on inflammatory mediators like Interleukin (IL-6, IL-8) and prostaglandin E_2 (PGE_2) produced by gingival fibroblasts stimulated by the LPS of *Aggregatibacter actinomycetemcomitans*. The LPS-induced IL-6, IL-8, and PGE_2 responses of gingival fibroblasts were inhibited by treatment with the cranberry fraction. This fraction was found to inhibit fibroblast intracellular signaling proteins, a phenomenon that may lead to a down-regulation of activating protein-1 activity. Cranberry components also reduced cyclooxygenase 2 expression. This study suggested that cranberry juice contains molecules (Type A linked PAC) with interesting properties for the development of new host-modulating therapeutic strategies in the adjunctive treatment of periodontitis.

La et al[24] investigated the effect of various concentrations of grape seed PAC on MMP secretion by human monocyte-derived macrophages which were stimulated with *Aggregatibacter actinomycetemcomitans* LPS. The secretion of MMPs and activation of nuclear factor-kappa B (NF-κB) p65 and activator protein-1 (AP-1) were assessed. GSE inhibited the secretion of MMP-1, -3, -7, -8, -9, and -13 by LPS-stimulated macrophages in a concentration-dependent manner. The suppression of MMP secretion was associated with inhibition of NF-κB p65 and AP-1 activation. Also, GSE dose-dependently inhibited the activity of MMP-1 and -9.

La et al [25] examined the effect of cranberry derived proanthocyanidins type A (AC PACs) on various MMPs stimulated with LPS of *Aggregatibacter actinomycetemcomitans*. AC-PACs inhibited the production of MMPs in a concentration-dependent manner. The catalytic activity of MMP-1 and MMP-9 was also inhibited. The inhibition of MMP production was associated with reduced phosphorylation of key intracellular kinases and the inhibition of NF-κB p65 activity. The study concluded that AC-PACs showed potential for the development of novel host-modulating strategies to inhibit MMP-mediated tissue destruction during periodontitis.

La et al[26] in 2010 further investigated the effects of AC-PACs on various virulence determinants of *Porphyromonas gingivalis* as well as on the inflammatory response of oral epithelial cells stimulated by P.gingivalis. The investigators observed that that while AC-PACs neutralized all the virulence properties of *P. gingivalis* in a dose-dependent fashion, they did not interfere with growth. They also inhibited the secretion of IL-8 but did not affect the secretion of IL-6 by epithelial cells stimulated with *P. gingivalis*. This further promises

AC-PACs to be a potentially valuable bioactive molecule for the development of new strategies to treat and prevent *P. gingivalis*-associated periodontal diseases.

PAC extracted from grape seeds has been shown to provide a significant therapeutic effect on *Escherichia coli* induced experimental periodontitis in rats.[27] The protective action of different doses of PAC was investigated in blood by assaying the various ROS and the antioxidant levels. PAC at an effective dose of 30mg / kg body weight, sc, for 30 days resulted in decrease in serum ROS, lipid peroxides, lysosomal enzymes, acute phase proteins and an increase in antioxidant levels. The PAC treated groups demonstrated only scattered inflammatory cells and blood vessels. The results showed that dietary supplementation of proanthocyanidin enhanced the host resistance as well as the inhibition of the biological and mechanical irritants involved in the onset of gingivitis and the progression of periodontal disease.

PACs are able to produce antiadhesive actions against bacteria in dental infections, including *Escherichia coli* and *Streptococcus mutans*. Their role in defense against LPS induced periodontitis has also been proved.[28] Irrespective of the source of PAC extract, it can be concluded that the mechanisms of action of PACs include the inhibition of (i) bacterial and host-derived proteolytic enzymes, (ii) host inflammatory response, and (iii) osteoclast differentiation and activity. Hence, proanthocyanidins should be considered as beneficial molecules in preventing or modifying the pathophysiology of many diseases and conditions. Further investigations and clinical trials are essential to understand the regulatory genes and pathways involved in their effects. This phytochemical can be considered as the new "natural" weapon involved in host modulation therapy for periodontal diseases.

CONCLUSION

Compounds that may have properties which inhibit biofilm development are important to study because they are potential natural remedy. Based on the results of previous studies, we believe more research should be focused on natural extracts as effective host modulation tools for chronic inflammatory diseases like periodontitis. Continuing research on the effects of different PAC compounds on biofilm inhibition would be beneficial. Biofilms are responsible for 65% of nosocomial infections. Since type A linked PAC has additional properties of anti adhesion, the focus can be streamlined to dose related strategies targeting the bacterial biofilm.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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