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Cancer Chemoprevention by Dietary Proanthocyanidins

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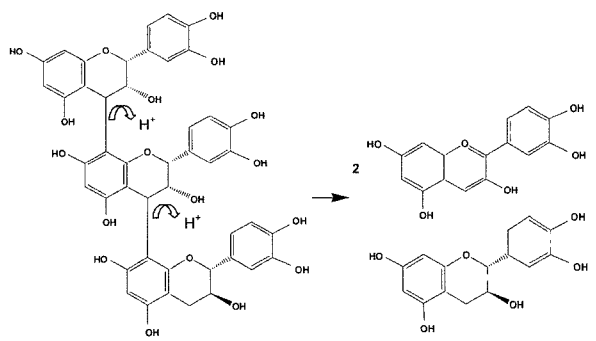
Abstract Proanthocyanidins (PACs), also named condensed tannins, are polymers of flavan-3-ols such as (+)-(gallo)catechin and (-)-epi(gallo)catechin. A proper analysis of the PACs, with difficult challenges due to their complex structures, is crucial in studies of cancer chemoprevention. Cancer is a leading cause of mortality around the world. Many experimental studies have shown that dietary PACs are potential chemopreventive agents that block or suppress against multistage carcinogenesis in both *in vitro* and *in vivo* models. Cancer chemoprevention by dietary PACs has been shown effective through different mechanisms of action such as antioxidant, apoptosis-inducing, and enzyme inhibitory activities. Good sources of dietary PACs are nuts, fruits, beans, chocolate, fruit juice, red wine, and green tea. The chemopreventive potential of dietary PACs should be considered together with their bioavailability in humans. The safety issues regarding carcinogenesis and gastrointestinal disorder are also reviewed.

Keywords: proanthocyanidin, chemoprevention, anticancer, condensed tannin, grape seed extract

Introduction

Proanthocyanidins (PACs), often called condensed tannins, are the most common tannins in our diet. They are found in nuts, fruits, beans, chocolate, red wine, and green tea. The term 'tannin' is derived from its capacity to precipitate protein and make leather from animal skin. PACs are polymeric flavonoids. Flavonoids are derived from claisen condensation of chalcone, which is synthesized via condensation of *para*-coumarate and 3 units of malony-CoA by chalcone synthase. The term 'proanthocyanidins' comes from the observation that these polymers yield anthocyanidin pigments upon oxidative cleavage (not hydrolysis) in hot alcohol (1) as shown in Fig. 1.

Many experimental studies have shown that dietary PACs are potential chemopreventive agents that block or suppress against multistage carcinogenesis in both *in vitro* and *in vivo* models. The purpose of this review is to introduce the previous works on PACs regarding their chemical structure, chemopreventive activities, dietary source, intake and bioavailability, and safety issues.

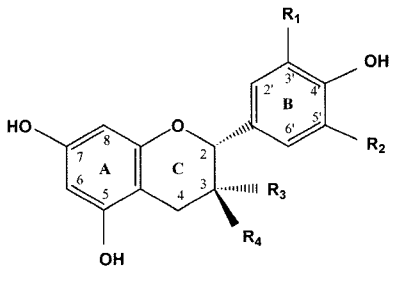


Procyanidin (epicatechin-(4β→8) -epicatechin-(4β→8)-catechin) 2 cyanidin+catechin (end group)

Fig. 1. Oxidative cleavage of proanthocyanidins (from Ref. 1).

Chemical Structure

PACs are polymeric flavan-3-ols, which are linked by C-C and sometimes by C-O-C bonds. The flavan-3-ol unit has the typical flavonoid scaffold, of which nomenclature system is defined with letters and numbers (Fig. 2). Although the biosynthetic pathways to flavonoids are well understood, it has not been well investigated on how they condense and polymerize to PACs. The most well known flavan-3-ol units are (-)-epicatechin and (+)-catechin. Addition of another hydroxyl group on the B ring of epicatechin and catechin makes epigallocatechin and gallocatechin, respectively. Flavan-3-ols with only a single hydroxyl group on the B ring are much less common. Upon oxidative cleavage in hot alcohol, procyanidins (catechin and epicatechin-based polymers) yield cyanidins; prodelphinidins (gallocatechin



	Flavan-3-ol monomers	R_1	R_2	R_3	R_4
Procyanidin	Catechin	OH	H	H	OH
	Epicatechin	OH	H	OH	H
Prodelphinidin	Gallocatechin	OH	OH	H	OH
	Epigallocatechin	OH	OH	OH	H
Propelargonidin	Afzelechin	H	H	H	OH

Fig. 2. Chemical structures of common flavan-3-ol monomers of dietary proanthocyanidins.

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and epigallocatechin-based polymers) yield delphinidins; and propelargonidins (mono-substituted flavan-3-ol based polymers) yield pelargonidins. Leucoanthocyanidins, which are easily confused with PACs, are the monomeric flavonoids, such as flavan-3,4-diols and flavan-4-ols, that yield anthocyanidins upon acidic treatment in heat and room temperature, respectively. Although they have similar reaction to PACs, they do not form precipitable complexes with protein (1).

The most common interflavanol linkages are C-C bonds between the C4 of the upper unit and C8 or C6 of the lower unit. These PACs are called B-type (dimeric) and C-type (trimeric) PACs. Compounds with doubly linked units (one C-C and one C-O; A type) have also been reported in cinnamon, plums, and cranberries (2, 3). In addition to the usual C4-C8 or C4-C6 linkage, these A-type PACs have an additional C-O bond between the C2 of the upper unit and the oxygen bound to C7 or C5 of the lower unit. The chemical structures of common A-type PACs are shown in Fig. 3. The direction of interflavanol linkage is described in parentheses with an arrow, and 4→ shows the configuration at C4. The common dimers, B1 to B8, are shown in Fig. 4. The most common PACs found in edible plants are procyanidins (PCs) and prodelphinidins (PDs). In PCs and PDs, 4→8 linkages are stereochemically favored, and usually, 4→8 and 4→6 linkages are present in a ratio of 3:1 (4). The most common trimeric PACs are known to be linear 4→8 polymers such as C1 and C2 found in grapes and barley, respectively (Fig. 5).

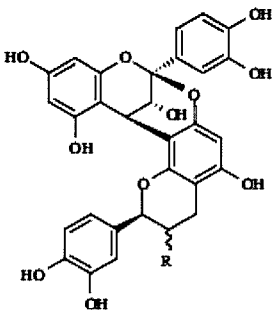

Fig. 3. Chemical structures of common A-type proantho-cyanidins.

Challenges to Analysis of Polymeric PACs

The degree of polymerization (DP) of PACs, which varies with the species or tissues, can be detected differently according to methods of extraction (5). For example, PACs with a high DP were reported to be better extracted by aqueous acetone than aqueous methanol (6). The anti-tumor-promoting effects of grape seed PACs with various DPs were much more potent than green tea polyphenols of mostly monomers (7). Therefore, analysis of the complex PACs is particularly crucial in studies of chemoprevention.

The analysis of polymeric PACs in any sample is a complicated issue for many reasons. First, the extraction of

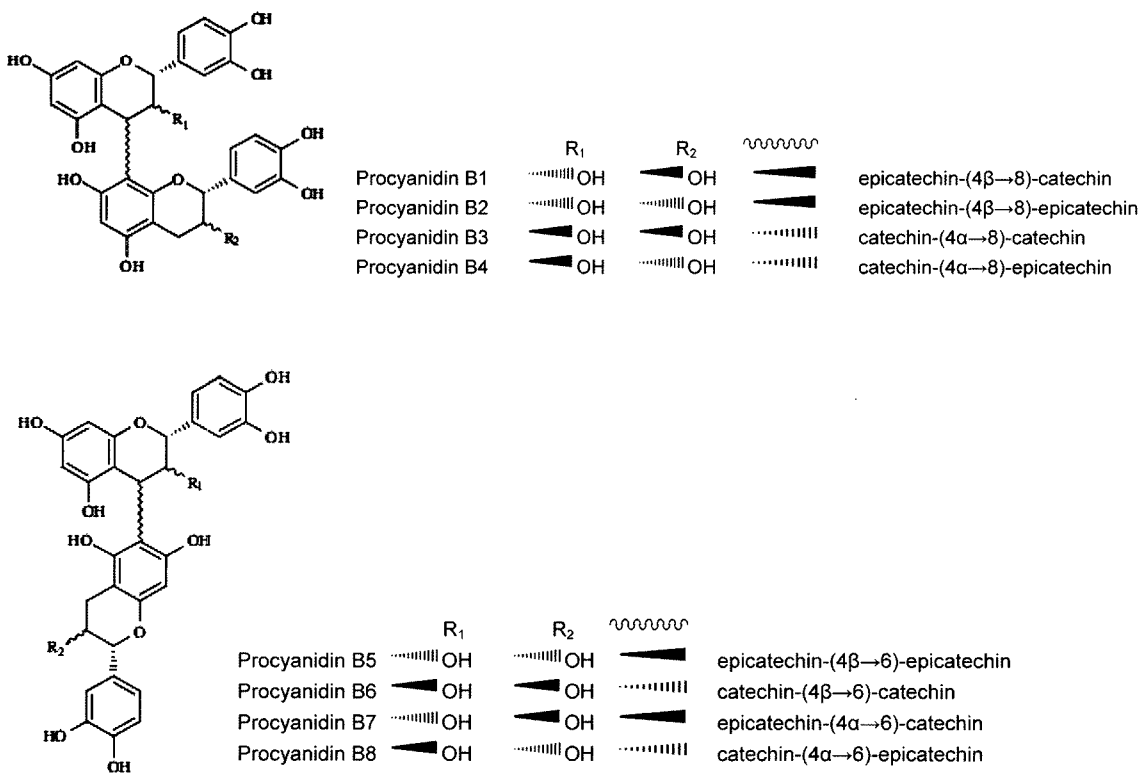

Fig. 4. Chemical structures of common B-type proantho-cyanidins.

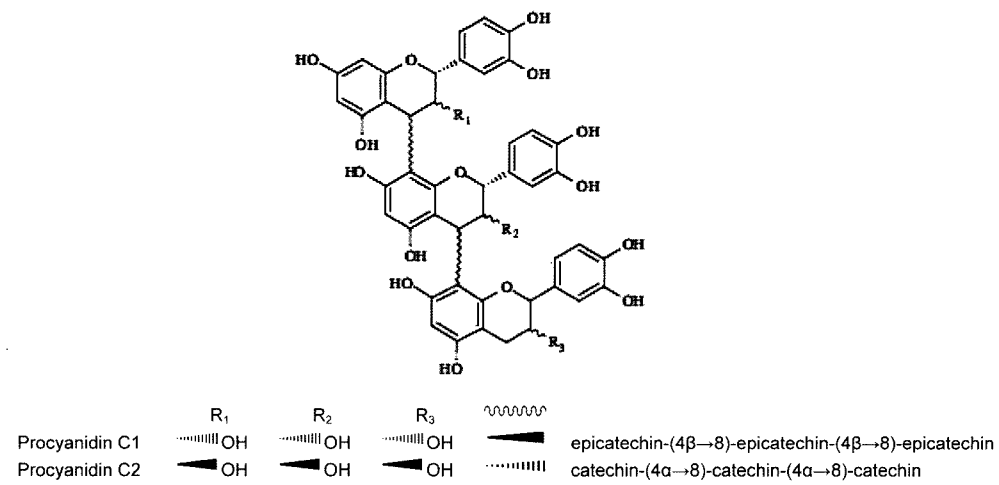

Fig. 5. Chemical structures of common C-type proanthocyanidins.

PACs is complicated by their strong interaction with proteins (8) and polysaccharides (9). PACs with a high DP are usually more strongly absorbed on silica matrix than PACs of a lower DP (10). Therefore, relatively inert matrix such as Toyopearl or Sephadex LH-20 has been successfully used to elute bioactive PACs of various DPs (11-13). The second complexity issue is due to the large number of diverse structures of PACs. Due to a wide range of polarities as well as the molecular weight ranges of PACs in grapes and their cultures, the separation of PACs is more challenged as the molecular size increases beyond oligomers (8). Although LC-mass spectrometry (MS) and the multiple mass spectrometry (MS/MS and MS_n) developed in the 90's have been applied to characterize the complex PACs (14), none of these can be considered totally satisfactory. For preliminary characterization of PAC-rich fractions, LC-ESI (electrospray ionization)/MS has been used widely. This method can detect mainly molecular ions due to soft ionization, and thus can be appropriate for characterization of thermolabile complex PACs (15-17), which are often co-eluted as a large peak (13, 18).

Chemopreventive Activities

Cancer and chemoprevention Cancer is a leading cause of mortality around the world. Every year, over 7 million people die of cancer, which is 12.5% of total deaths worldwide. More than 70% of all the cancer deaths occur in low- and middle-income countries, where health care resources for prevention, diagnosis, and management are very limited (19). Over 40% of cancer is possibly prevented by eating a healthy diet, exercising regularly, and quitting (or not starting) smoking. In Korea, cancer has been the largest cause of mortality for over 20 years (20), and the mortality rate has been rising for the last decade due to dramatic increases in risk factors such as tobacco use, a Westernized diet and a sedentary lifestyle. In the United States, cancer is the second largest cause of mortality exceeded only by heart diseases, and the mortality rate has not been significantly lowered since 1950 (21). In Japan, cancer is the leading cause of death (22), and the mortality rate (222 deaths per 100,000 population) is higher than cancer death rates in USA (191 deaths) and Korea (135 deaths). Current trends in functional food research around the world reflect the crucial role of diet in cancer chemoprevention.

Carcinogenesis consists of 3 distinct steps - initiation, promotion, and progression (23). Initiation begins when normal cells are exposed to a carcinogen and their cDNA undergoes damage, which remains unrepaired or misrepaired. Promotion is a reversible process of active proliferation of damaged cells. Progression irreversibly produces a new clone of tumor cells that are proliferating, invading, and spreading to other tissues (metastasis). Chemopreventive agents inhibit, reverse or retard carcinogenesis.

There are 2 classes of chemopreventive agents. Blocking agents inhibit the tumor initiation stage. They inhibit metabolic activation of the procarcinogens to their ultimate carcinogens and their subsequent interaction with DNA. They also stimulate the detoxification of carcinogens, leading to their secretion from the body. Suppressing agents inhibit the malignant transformation of initiated cells in either the promotion or the progression stage (23). Many experimental studies have shown that dietary PACs are potential chemopreventive agents that block or suppress against multistage carcinogenesis.

***In vitro* cytotoxicity** (-)-Epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG), which are found in green tea, induced apoptosis, a programmed cell death, in cancer cells but not in normal cells (24-26). Galloylated procyanidin dimers also showed cytotoxicity against human melanoma, leukemia, and lung and colon cancer cell lines (27-29). The non-galloylated dimers, A-type dimers and trimers, and a galloylated pentamer, however, were less active, suggesting structure-activity relationships of PACs regarding cytotoxicity to human cancer cells.

Grape seed extracts with 75-80% oligomeric PACs showed cytotoxicity to numbers of cancer cells including human breast cancer, lung cancer, and gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal and murine macrophage cells (30, 31). Likewise, the selective cytotoxicity of PACs to cancer

cells was also demonstrated by Jo *et al.* (13). The PAC-rich fraction from grape cell culture extracts showed significant cytotoxicity to human hepatoma and mouse leukemia cells, without showing any cytotoxicity to non-cancerous pig kidney cells. After further subfractionation of this chemopreventive fraction using Sephadex LH-20, the cytotoxicity of bioactive subfractions to human hepatoma cells was found to be proportional to the DP of PACs in each mixture.

***In vivo* chemoprevention** Dietary PACs showed the protective effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced risks including skin tumor promotion, and hepatic and brain lipid peroxidation, and DNA fragmentation in mice (7, 32, 33). A significant (72-88%) inhibition of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) formation was also observed in female rats fed with diets containing 0.1-1.0% grape seed PACs (34). These rats also showed a 20-56% inhibition of ornithine decarboxylase (ODC) activity in the distal third of the colon, suggesting that PACs were highly bioavailable in colon and inhibited carcinogenesis at promotion stage partly by ODC inhibition. Kim *et al.* (35) found that orally given grape seed PACs significantly (50%) inhibited 7,12-dimethylbenz(a)-anthracene (DMBA)-induced breast tumor formation in rats.

Mechanisms of action Cancer chemoprevention by dietary PACs has been shown effective through different mechanisms of action, mainly antioxidant, apoptosis-inducing, and enzyme regulating activities.

The chemopreventive activities of PACs have been linked to their antioxidant capacities by Bagchi *et al.* (36). The free radical scavenging ability of PACs was significantly better than vitamins C, E, and β-carotene in both *in vitro* and *in vivo* models. Under certain conditions or at higher doses, various classes of polyphenols including PACs showed cytotoxicity through prooxidant capacities such as free radical generation or oxidative DNA cleavage either alone or in the presence of metals such as Cu(II) and Fe(III) complexes (37-39).

The cytotoxicity of PACs to various cancer cells was associated with apoptosis, through nitric oxide (NO) production (40), H_2O_2 production (41), mitogen-activated protein kinases (MAPK) and caspase-3 activation (42), nuclear factor kB (NF-κB) inhibition (43), and cki (cyclin kinase inhibitor)-cyclin-cdk (cyclin-dependent kinase) machinery modulation (44).

The target enzymes for chemopreventive inhibition by PACs include ODC (11, 33, 34), protein kinase C (33), and human DNA topoisomerase (topo) I and II (12, 45). ODC, a key enzyme in polyamine biosynthesis, is highly expressed at initiation and promotion stages. Protein kinase C is involved in cell signaling and tumor promotion. DNA topo I and II, essential enzymes for cell division and cell proliferation, also have emerged as chemotherapeutic targets for a diverse group of anticancer agents including camptothecin and etoposide (VP-16). Both ODC and topo II are known to be expressed highly in proliferating tumor cells (46-48), which makes these enzymes clinical targets for selective chemoprevention. Previous studies have shown that the enzyme inhibitory activities of PACs were also positively related to their DP (5, 13). This suggests eating fruits and nuts rich in PACs of various DPs may be more beneficial to health in cancer chemoprevention than drinking tea mainly of PAC monomers.

Other mechanisms of action include inhibition of cyclo-oxygenase (COX)-2 expression rather than the enzyme activity (49, 50), inhibition of DNA methyltransferase (51), and protection of gap-junction intercellular communication (GJIC) inhibition (52).

Dietary Sources, Intake, and Bioavailability

Dietary sources Good sources of dietary PACs are nuts, fruits, beans, chocolate, fruit juice, red wine, and green tea (3, 5, 53, 54). The data for the PAC content per standard serving size of these foods are summarized in Table 1. The largest PAC content per serving size was found in nuts followed by fruits like plums, berries, and apples.

Dietary intake and bioavailability The estimated daily intake of PACs in the United States is about 57.7 mg per person over 2 years old (3). The consumption of monomers, dimers, trimers, and those above trimers were estimated 7.1, 11.2, 7.8, and 73.9% of total PACs, respectively. Due to higher consumption of green tea (200 mg of catechins per serving) in Asian countries like Korea, Japan, China, and India, the amount of total PAC intake may be higher in these populations.

The chemopreventive potential of dietary PACs should be considered together with their bioavailability in humans. An *in vitro* study showed that radiolabelled (+)-catechin and PAC dimer and trimer were absorbed through human Caco-2 cell monolayer with similar permeability coefficients close to that of mannitol, a hydrophilic marker of medium permeability (55). PAC polymers (average DP = 6), however, showed 10 times lower permeability in the study. Another *in vitro* study showed that PAC polymers were degraded by human colonic microflora into low-molecular weight phenolic acids, such as hydroxylphenyl-acetic, hydroxylphenylpropionic, and hydroxylphenylvaleric acids (56), suggesting that only these metabolites may be absorbed through the gut barrier after a PAC polymer-rich diet (57).

Very few *in vivo* studies regarding bioavailability of PACs have been carried out. It is due to the structural complexity of these compounds and a lack in pure commercial standards. The PAC monomers are moderately absorbed in adults when compared to highly absorbed isoflavonoids or poorly absorbed anthocyanins (58). PAC dimer B2 was also detected in human plasma after consumption of a cocoa (59). Orally given grape seed PACs increased urinary excretion of phenolic acids, including 3-hydroxyphenyl-propionic acid, 4-*O*-methylgallic acid, and 3-hydroxyphenylacetic acid, suggesting that these are major metabolites of PAC metabolism in human (60).

Based on the estimated daily intake and absorption rate for PACs, the plasma concentration of intact PACs may be as low as nanomolar levels, which is much lower than micromolar levels of *in vitro* chemopreventive doses. However, orally given PACs showed cytoprotection at multiple target organs (liver, kidney, heart, and brain) in

Table 1. Proanthocyanidin content of selected foods[1)]

Foods	mg per serving size[2)]							
	DP[3)]							
	1	2	3	46	710	>10	Total	Type[4)]
Fruits								
Apple, *Gala,* with peel	9	15	9	32	29	48	143	PC
Apple, *Fuji,* with peel	9	15	9	29	22	22	106	PC
Blueberries	2	4	3	15	11	94	129	PC, PD
Cranberries	3	11	8	30	27	102	182	PC, A
Grapes, red	1	2	1	5	5	36	49	PC, PD
Grapes, green	1	2	2	6	7	47	65	PC, PD
Peaches, yellow, with peel	3	10	3	15	9	18	59	PC
Pears, green, with peel	2	2	2	5	4	20	35	PC
Plums, black, with peel	7	17	16	47	32	87	205	PC, A
Plums, with peel	9	32	18	48	28	47	183	PC, A
Strawberries	3	4	4	23	20	63	118	PC, PD, PP
Beans								
Beans, pinto, cooked	9	18	18	50	18	5	117	PC, PP
Nuts								
Almonds	9	12	11	47	44	94	216	PC, PP
Hazelnuts	12	15	16	80	88	377	587	PC, PD
Pecans	20	49	30	118	98	261	577	PC, PD
Pistachio nuts	13	15	13	49	44	143	277	PC, PD
Walnuts	8	7	8	26	6	23	78	PC
Peanuts	6	5	5	4	0	0	19	PC, A
Beverages								
Apple juice	1	1	1	0	0	0	3	PC
Cranberry juice cocktail	0	1	0	1	1	NA[5)]	4	PC, A
Grape juice, purple	0	1	0	2	1	7	12	PC, PD
Tea*	2	1	0	NA	NA	NA	3	PC, PD
Wine, red	3	3	0	1	1	2	9	PC, PD
Sweets								
Chocolate, dark*	32	24	13	19	6	NA	93	PC
Chocolate, milk	9	9	6	16	7	13	61	PC

[1)]Modified from Ref. 3, 5, 53, and 54; Data with asterisks (*) show a relatively low reliability of proanthocyanidin content.
[2)]The serving sizes of the selected foods are as follows: apples (1 medium, 154 g), blueberries (½ cup, 73 g), cranberries (½ cup, 48 g), grapes (1½ cup, 80 g), and the other fruits (½ cup, 83 g), beans (1½ cup, 450 g), nuts (1 cup, 117 g), non-alcoholic beverages (8 fl. oz., 240 mL), wine (5 fl. oz., 150 mL), and chocolates (1 bar, 40 g).
[3)]Degree of polymerization.
[4)]PC, procyanidins; PD, prodelphinidins; PP, propelargonidins; and A, A-type linkages.
[5)]Not applicable.

mice, demonstrating high bioavailability of these nutraceuticals (36). Considering other reports on the discrepancy between *in vitro* (10-100 μM) and *in vivo* chemopreventive doses (0.1 μM) of orally taken resveratrol, it is possible that a chemopreventive health benefit from a PAC-rich diet may be achieved at much lower levels of intake than levels suggested from the *in vitro* assays. Enterohepatic circulation of bioactive metabolites may also explain the discrepancy between effective PAC doses *in vitro* versus in the diet.

Safety Issues

Carcinogenesis issue The prooxidative effects of PACs

have been reported under certain *in vitro* conditions or at higher doses, and thus, may not occur *in vivo* from a normal intake of PAC-rich foods (39). For example, PAC-rich extracts have been reported to be carcinogenic when injected subcutaneously to rats, but their carcinogenic effect was not proven when orally given (5). The association of the consumption of PAC-rich betel nuts with esophageal cancer in humans has raised more safety issue of dietary PACs. This can be explained not only by exceedingly high amount of PACs in betel nuts, but also by the nut-chewers' life style (social class, smoking, etc.) or by a large amount of alkaloids which can be responsible for the risk (5). PAC-rich sorghum was claimed to be associated with esophageal cancer, but ecological studies showed a low risk of esophageal cancer in regions where sorghum is a main food source. Several epidemiological studies showed a positive association between the consumption of tea and esophageal cancer, but it was explained later that the high temperature of hot tea, not PACs and other polyphenols in tea, caused esophageal cancer (61). The discrepancy on the safety issue of PAC-rich diet, therefore, can be explained by dose-response effects, metabolic inactivation, or life style factors (5, 62). In fact, a clearly protective effect of green tea consumption against esophageal and lung cancer could be observed in nonsmokers only (63, 64).

Gastrointestinal disorder issue Traditionally, PACs (condensed tannins) have been considered to reduce digestion and absorption due to their binding to several macronutrients in ruminants. Currently, however, the protein-binding traits of PACs are considered to have more specific digestive advantages in ruminants (65, 66). PACs slow down dietary protein digestion in the rumen, preventing bloating and improving the animal's nitrogen nutrition. This property may also be potentially useful in preventing obesity in humans as suggested by Awika and Rooney (67).

Future Research

Future *in vitro* chemoprevention studies should be focused more on the metabolites of dietary PACs than intact PAC themselves, in order to understand their potential as chemopreventive agents. Knowledge of drug-supplement interaction will be beneficial for safer and more effective cancer treatment. Also, we need more *in vivo* or human clinical studies in order to obtain better knowledge of anticancer activities by dietary PACs. The amounts of PACs, which are thermolabile, in various cooked or heat-treated foods and beverages are also to be analyzed to gauge any possible health benefit in our daily diet. Data on PAC intake by various ethnic groups or in culturally distinct regions in the world are not available at this point. We also need to know any possible long-term side effect due to the large consumption of PAC-rich foods or supplements (e.g., grape seed extracts) in humans.

Conclusions

The worldwide consumption of a PAC-rich diet such as nuts, fruits, and beans creates the possibility of exploiting the properties of dietary PACs as chemopreventive agents. Many experimental studies have shown that PACs are potential chemopreventive agents that block or suppress against multistage carcinogenesis in both *in vitro* and *in vivo* models. Their mechanisms of action include antioxidant, apoptosis-inducing, and enzyme inhibitory activities. The chemopreventive potential of dietary PACs, however, should be considered with their bioavailability in humans. Further *in vitro* chemoprevention studies with dietary PACs are necessary with more attention being paid to their metabolites rather than intact compounds alone. Moreover, we need more *in vivo* or human clinical studies in order to obtain a better knowledge of anticancer activities by these dietary PACs. Also, possible long-term adverse effects due to the high consumption of a PAC-rich diet or supplements, and potential drug-supplement interaction, should be fully investigated before any clinical claim of health benefits by these nutraceuticals.

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