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Review Article

Antiinflammatory and antitumor effects of flavonoids and flavanoids

Jen-Kun Lin[1*], Shu-Huei Tsai[1] and Shoei-Yn Lin-Shiau[2]

Institutes of [1]Biochemistry and [2]Pharmacology, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-ai Road, Taipei, Taiwan.
[*]Correspondence

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Introduction

Phenolic compounds are widespread in the plant kingdom. The major groups of phenolic compounds are the flavonoids and flavanoids, which are important in contributing the flavor and color of many fruits and vegetables. They are also important for the normal growth, development and defense of plants (1, 2). Interest has recently been focused on flavonoids and flavanoids because of their broad pharmacological activities, and their daily human intake has been estimated to be more than 1 g with the primary dietary source being vegetables and fruits (3). They also are found in several medicinal plants, and herbal remedies containing flavonoids and flavanoids have been used in folk medicine throughout the world (4). It has been reported that diets rich in fruits and vegetables appear to protect against cardiovascular disease and some forms of cancer (5-7).

The important group of phenolics in food are flavonoids and flavanoids, which consist mainly of flavones, flavanones, flavonols, flavanonols, isoflavones, flavanols and anthocyanidins (Fig. 1) (8). Although flavanols, also called catechins, seem to be widely distributed in plants, they are rich only in tea leaves, where they may constitute up to 30% of dry leaf weight. The antioxidative and antitumor properties of green and black teas and their tea polyphenols are well documented. Since flavonoids and flavanoids appear to be important not only for plants but also for humans, this brief review will discuss the current data with a particular emphasis on the antioxidant, antiinflammatory and antitumor properties of flavonoids and flavanoids.

Chemistry and biosynthesis

Flavonoids are biosynthesized via a combination of the shikimic acid and acrylpolymalonate pathways. Through subsequent hydroxylations and reductions, plants are able to synthesize different classes of flavonoids (9). They are C_{15} compounds of 2 phenolic rings connected by a 3-carbon unit, and they can be grouped according to the presence of different substituents on the rings and to the degree of ring saturation. They are frequently attached to sugars (glycosides) and as such tend to be water-soluble. Since more than 4000 flavonoids have been found, in this study we will only discuss the biological function of three family members of flavonoids including flavanones (*e.g.*, naringenin, taxifolin and fustin), flavones (*e.g.*, apigenin, luteolin and chrysin) and flavonols (*e.g.*, fisetin, quercetin, kaempferol, myricetin and galangin).

Flavonoids

Antioxidant activity

Free radicals, such as singlet oxygen, superoxide radical, hydrogen peroxide, hydroxy radical, nitric oxide and peroxynitrite, are highly unstable and extremely reactive. The wide distribution of these species makes them highly toxic to tissues. On the other hand, free radicals are continuously produced in the body during normal metabolism (10). Because of the potential damaging nature of free radicals, tissues possess a number of antioxidant

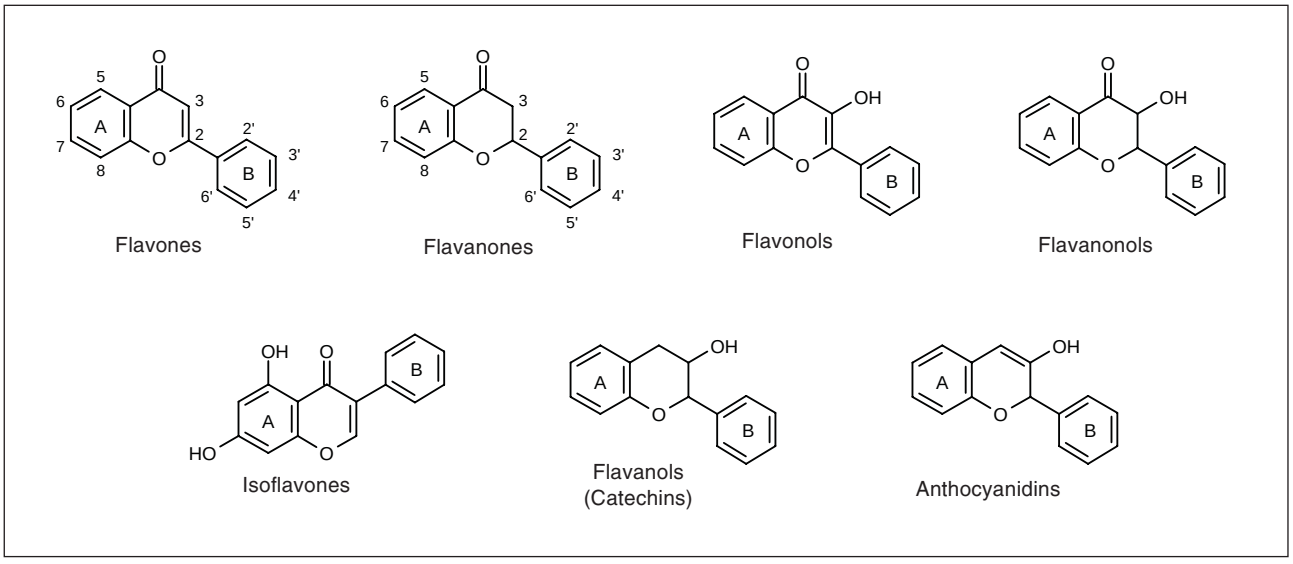

Fig. 1. General chemical structures of flavonoids, flavanoids and anthocyanidins. Flavonoids comprise flavones, flavonols and isoflavones, while flavanoids comprises flavanones, flavanonols and flavanols.

defense mechanisms that include a system of enzymatic and nonenzymatic antioxidants and reactive oxygen species (ROS) scavengers. Three of the most important cellular antioxidant enzymes are superoxide dismutase, glutathione peroxidase and catalase. In addition, flavonoids can act as antioxidants through a number of potential signaling pathways. The most important is likely to be by free radical scavenging, in which the polyphenol can break the free radical chain reaction.

The main structural features of flavonoids required for efficient radical scavenging effects have been reported. Hydroxyl groups at position 3 and 5 provide hydrogen bonding to the keto group and an *ortho*-dihydroxy structure in the B ring offers electron delocalization (11). In addition, the 3-OH group in combination with a C2-C3 double bond increases the scavenging activity. Another pathway for the antioxidant effect of the flavonoids is mediated by chelating metal ions. Metal ions, especially the transition metals such as Fe^{2+} and Cu^{2+}, are the key components involved in Fenton-type reactions, which can generate highly reactive hydroxyl radicals (12).

It should be pointed out that oxidative stress may be induced when antioxidant defenses are unable to cope with the free radicals produced from the action of certain toxins and physiological stress (13). Using the oxygen radical absorbance capacity assay, Cao *et al.* clearly demonstrated that the same flavonoids could behave as both antioxidants and prooxidants, depending on concentration and free radical source. Flavonoids acted as antioxidants against free radicals but demonstrated prooxidant activity when a transition metal was available (14). The copper-initiated prooxidant activity of flavonoids, as its peroxyl or hydroxyl radical absorbing antioxidant activity, also depends on the number of free hydroxyl substitutions on its structure. The more hydroxyl substitutions there are, the stronger the prooxidant activity is. Therefore, one question that must be addressed is whether these flavonoids are better antioxidants than other common antioxidants, such as α-tocopherol and ascorbic acid. It has been suggested that flavonoids are as good as or better than α-tocopherol and ascorbic acid in terms of their antioxidant activity; transition metal-induced prooxidant actions of ascorbic acid and α-tocopherol have been well studied (15-18). Although the copper-initiated prooxidant actions of flavonoids and other antioxidants including ascorbic acid and α-tocopherol may not be important *in vivo*, copper ion will be largely sequestered, except perhaps in certain metal overload diseases. However, the prevention of iron-increased lipid peroxidation in hepatocytes by some flavonoids including quercetin has been reported (19, 20).

Antiinflammatory activity

One of the most important side effects of conventional antiinflammatory drugs is their ulcerogenic activity on the gastrointestinal tract. In contrast, in some reports flavonoids were shown to possess *in vivo* antiinflammatory properties without the ulcerogenic side effects. In fact, flavonoids were also able to protect the gastric mucosa against a variety of ulcerogenic agents (21). Many studies were carried out to define the possible mechanisms for the antiinflammatory activity of flavonoids. Both prostaglandin and nitric oxide (NO) are crucial inflammatory mediators and play an important role in inflammatory processes. Cyclooxygenase (COX), the key enzyme in prostaglandin biosynthesis, exists in two isoforms, COX-1 and COX-2. These two COX isoforms share about 60% sequence similarity at the amino acid level, and the residues that are critical for enzyme function in both isoforms are highly conserved (22). However, COX-2

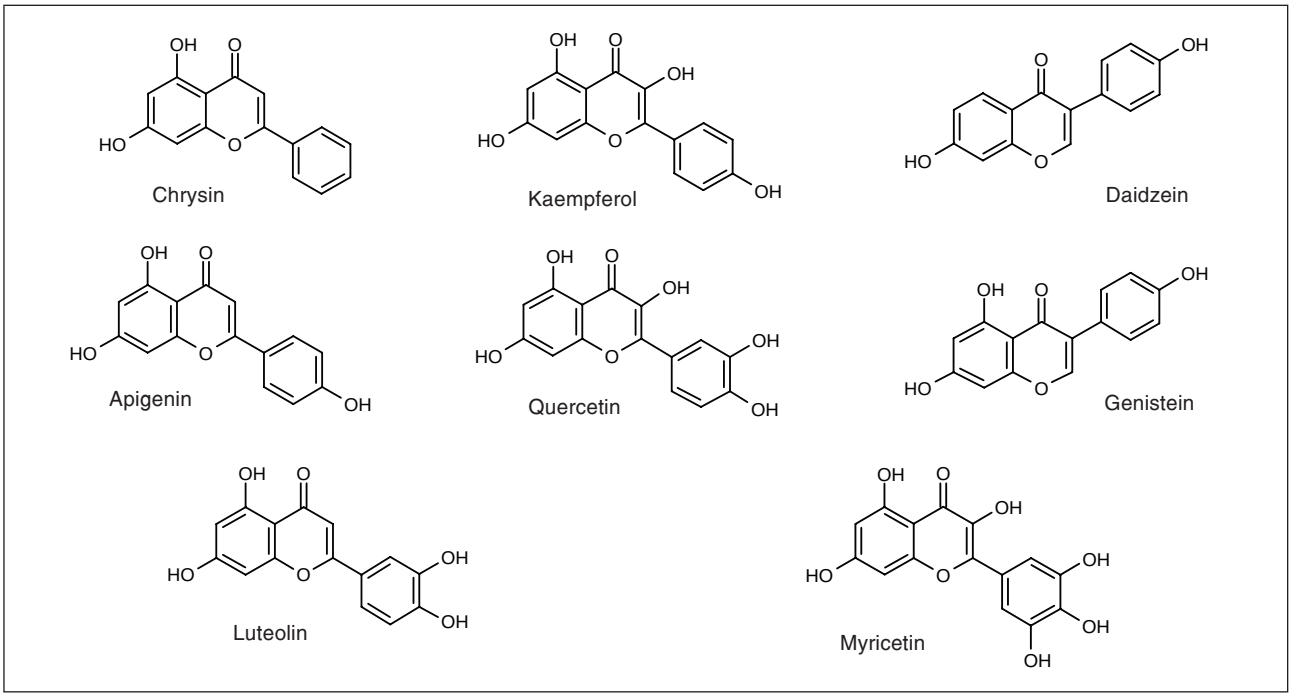

Fig. 2. Chemical structures of representative flavonoids, classified into three groups: flavones (chrysin, apigenin, luteolin), flavonols (kaempferol, quercetin, myricetin) and isoflavones (daizein, genistein).

expression is regulated by mechanisms different from COX-1 expression. COX-2 is not expressed under physiological conditions in most organs but is induced by cytokines and mitogens during inflammatory processes (23). The inhibition of COX appeared to be one of the important mechanisms involved in the antiinflammatory effects of flavonoids, such as flavone and apigenin.

In view of the potential detrimental effect of high amounts of NO produced by inducible NO synthase (iNOS) in inflammation, prevention of the expression of this enzyme represents an important antiinflammatory activity of flavonoids. Apigenin, chrysin, narigenin, kaempferol and quercetin were all reported to inhibit protein and mRNA expression of iNOS (24-26). Molecular cloning and sequencing analyses revealed that there are at least 3 main types of NOS isoforms. Both neuronal (nNOS) and endothelial NOS (eNOS) are constitutively expressed (27), whereas iNOS is inducible in response to interferon-gamma, lipopolysaccharide (LPS) and a variety of proinflammatory cytokines. The inducible isoform of NOS is responsible for the overproduction of NO in inflammation (28).

Nuclear factor κB (NFκB) is a pleiotropic transcription factor with key functions in the immune system. NFκB family members control transcriptional activities of various promoters of proinflammatory cytokines, cell surface receptors, transcription factors and adhesion molecules that are involved in inflammation (29). The presence of the *cis*-acting NFκB element has also been demonstrated in the 5'-flanking regions of both COX-2 and iNOS genes (30, 31). NFκB can be found in the cytoplasm of most cells as an inactive complex with unprocessed precursor proteins (*e.g.*, p105) or IκB (*e.g.*, IκBα) proteins. Activation of cells with various stimuli then initiates a signaling cascade that finally leads to the disruption of the inactive complex and the release of NFκB. Upon activation, NFκB translocates into the nucleus and binds to DNA, leading to activation of transcription (30). Apigenin and naringenin have been reported to inhibit NFκB activation by preventing the degradation of IκBα protein (24, 33). Furthermore, quercetin was found to inhibit LPS-induced NFκB complex p50/p50, but not complex p65/p50, as determined by mobility shift (21). Thus, apigenin, naringenin and quercetin could act as inhibitors of iNOS biosynthesis by downregulating the NFκB binding activity while chrysin represses iNOS gene expression by blunting the DNA-binding activity of interferon regulatory factor 1 (IRF-1), which is one of the important binding sites in the promoter region of iNOS gene, rather than by interfering with the NFκB binding activity (25).

Antitumor effects

Most flavonoids (Fig. 2) are diphenylpropanoids that occur ubiquitously in plant foods and are important constituents of the human diet. The major flavonoids are apigenin, quercetin, kaempferol, myricetin and luteolin. Although flavonoids are generally considered to be non-nutritive agents, interest in them has increased because of their potential role in the prevention of human cancer (34).

Apigenin and other related flavonoids inhibited carcinogen-induced tumors in rats and mice (35, 36). Apigenin, a less toxic and nonmutagenic flavonoid, suppressed 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-induced tumor promotion of mouse skin. TPA had the ability to activate protein kinase C (PKC) and induced nuclear proto-oncogene expression. Our recent studies indicated that apigenin inhibited PKC, probably by competing with ATP. Apigenin also reduced the level of TPA-stimulated phosphorylation of cellular proteins and inhibited TPA-induced *c-jun* and *c-fos* expression (37, 38).

Genistein is an isoflavone that occurs naturally in soybeans and some forage plants as the glucoside and has been identified in soy food products and beer. Epidemiological studies have indicated an association between a decrease in the incidence/mortality of hormone-dependent cancer (*e.g.*, breast and prostate) and a traditional soy-rich Asian diet (39). Individuals consuming this diet have 7- to 10-fold higher plasma and 30-fold higher urinary genistein concentrations than individuals consuming a typical Western diet. A tentative causal relationship is suggested by several studies showing the inhibitory effects of dietary soy products in chemical- and radiation-induced rat mammary and estrogen-induced mouse prostate models of carcinogenesis (40, 41).

Soy is a unique dietary source of the isoflavones, genistein and daidzein. Heavy consumption of soy in Southeast Asian populations is associated with a reduction in the rates of breast and prostate cancer and cardiovascular disease. The potential chemopreventive efficacy of genistein appears to be related to its phytoestrogenic effects (42). Genistein competes with estradiol for estrogen receptors and the complex translocates to the nucleus, stimulating estrogen-related cellular events and accelerating cellular differentiation (43).

Some estrogen-independent mechanisms of genistein are important for its biological action. Genistein significantly inhibits tyrosine-specific protein kinase activity (44), which may, in turn, inhibit cell proliferation and growth factor-stimulated responses and immune response, as well as induce cellular differentiation.

Flavanoids

Antimutagenic activity

The major flavanoids (Fig. 3) tea polyphenols potently decreased the mutagenicity of a number of aryl- and heterocyclic amines, of aflatoxin B_1, benzo[*a*]pyrene, 1,2-dibromoethane and of 2-nitropropane in *Salmonella typhimurium* (45). Recently, we also examined the antimutagenic properties of various tea extracts (green, pauchong, oolong and black teas) against different groups of environmental mutagens. The results suggest that different degrees of tea fermentation produce different types of antimutagenic compounds effective against their corresponding mutagens (46). Black tea polyphenols were more potent inhibitors of mutagenicity than green tea ones caused by the food mutagen PhIP in the *Salmonella* test system (47). Another study by Hernaez *et al.* suggested that catechin might protect against such diverse reactive intermediates as free radicals and electrophile forms during the metabolic activation of a cooked meat-derived mutagen (48).

Antioxidant activity

Tea flavanoids are potent scavengers against most ROS including superoxide, hydrogen peroxide, hydroxyl radicals and NO produced by various systems. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of various tea polyphenols were investigated (49). This activity was proportional to the number of hydroxyl groups in the catechins or theaflavins.

Recently, several studies have found that black tea and green tea offered protection against oxidative damage to red blood cells induced by a variety of inducers such as hydrogen peroxide, primaquine, phenylhydrazine and xanthine/xanthine oxidase system (50). Several reports indicated that tea intake may enhance the levels of antioxidant defense enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (51, 52). A previous study in our laboratory also found that after intraperitoneal injection of green tea polyphenols in rats, the antioxidant and phase II enzyme activities were elevated (53). Recently, we found that long-term feeding of powdered green tea leaves to rats resulted in enhanced SOD activity in serum and catalase activity and glutathione levels in the liver (54).

The inhibitory effects of 6 tea polyphenols, namely theaflavin, theaflavin-3-gallate, theaflavin-3,3'-digallate, (–)-epigallocatechin-3-gallate (EGCG), gallic acid and propyl gallate, on xanthine oxidase activity were investigated (55). Theaflavins and EGCG inhibit xanthine oxidase to produce uric acid and also act as scavengers of superoxide. Theaflavin-3,3'-digallate acts as a competitive inhibitor and is the most potent inhibitor of xanthine oxidase among these compounds. Tea polyphenols have potent inhibitory effects on TPA-stimulated superoxide production at 20-50 μM in HL-60 cells. It is suggested that the antioxidative activity of tea polyphenols is due not only to their ability to scavenge superoxide but also to their ability to block xanthine oxidase and related oxidative signal transducers (55).

Antiinflammatory activity

The induction of inflammation in skin mediated by TPA is believed to be governed by COX, lipoxygenase and ornithine decarboxylase (ODC). These markers of inflammatory response are important for skin tumor promotion. Application of black tea polyphenols prior to TPA application resulted in significant inhibition of TPA-caused induction of epidermal ODC and COX enzyme activities (56). NO radicals have a broad biological role in

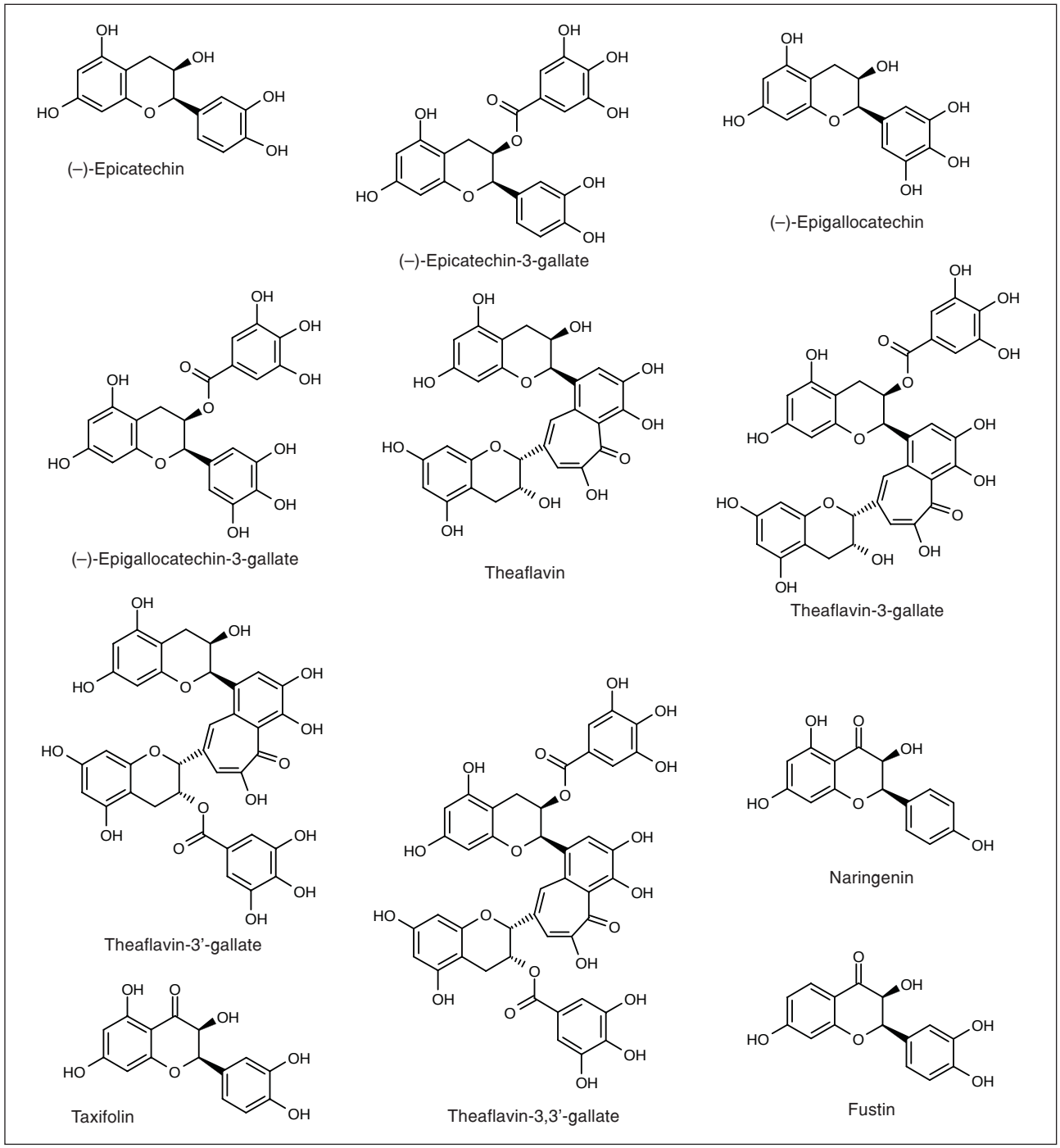

Fig. 3. Chemical structures of representative flavanoids, classified into two major groups: flavanones (naringenin, taxifolin, fustin) and flavanols, comprised of green tea polyphenols ((–)-epicatechin, (–)-epicatechin-3-gallate, (–)-epigallocatechin, (–)-epigallocatechin-3-gallate) and black tea polyphenols (theaflavin, theaflavin-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate).

modulating physiological and pathological processes (57). During infection and inflammation, the formation of NO is increased, which might promote carcinogenesis. Our studies showed that both EGCG and theaflavin-3,3'-digallate inhibited the induction of NO in thioglycollate-elicited and LPS-activated peritoneal or RAW264.7 macrophages (58, 59). Our results indiciated that EGCG decreased the activity and protein levels of iNOS by reducing the expression of iNOS mRNA, and that the reduction could occur as a result of preventing the binding of NFκB to the iNOS promoter, thereby inhibiting the induction of iNOS transcription (58).

Antitumor effects

Conney *et al.* were the first to demonstrate that hydroxylated flavanoids in tea had a potent inhibitory effect on mutagenic activity of diol epoxide of polyphenolic aromatic hydrocarbon (60). In recent years, many studies have demonstrated that topical application or oral feeding of a polyphenolic fraction from tea extract, and individual catechin derivatives, had anticarcinogenic effects in animal skin experiments (4, 61).

The inhibitory effects of tea polyphenols on carcinogenesis in experimental animal models have been recently reviewed (62). Tea polyphenols can suppress various chemically induced tumors in animal models, can inhibit tumor growth in established papilloma and can also inhibit the invasion and metastasis of established malignant tumors (63).

Tea polyphenols have profound chemopreventive activities in animal cancer models including colon, duodenum, esophagus, forestomach, liver, lung, mammary gland and skin (64).

Mechanisms of action of flavonoids and flavanoids

The potential application of flavonoids and flavanoids in medicine has primarily been focused on the chemoprevention of cancer and certain chronic diseases including cardiovascular diaseases, neurodegenerative disorders and immune diseases. The role of ROS in the pathogenesis of these chronic diseases has been emphasized. Therefore, it is conceivable that multiple mechanisms may be responsible for the chemopreventive activities of flavonoids and flavanoids, including removal of ROS from the site of production or the site of interaction and inhibition of lipid peroxidation and free radical formation, ODC, COX and PKC (64). The major flavanoids, tea polyphenols, suppress cellular proliferation through inhibition of epidermal growth factor receptor binding and autophosphorylation (65). Tea polyphenols also inhibit carcinogen-DNA binding and adduct formation by modulation of cytochrome p450 enzymes and induce phase II enzymes such as glutathione S-transferase, glutathione peroxidase, catalase and NADPH-quinone reductase (53).

The chemoprevention by tea polyphenols through mitotic signal transduction blockade has been critically and intensively discussed (66). Based on this contention, most chronic diseases, such as cancer and cardiovascular diseases, can be enhanced by cellular ROS and oxidative mitotic signal transducers, and these oxidative stresses or stimuli can be suppressed or attenuated by tea polyphenols and other flavonoids through signal transduction blockade (66).

Conclusions

In light of the pleiotropic effects of flavonoids and flavanoids, we have addressed their important effects on various biological systems. They could possess potent and desirable biological activities against cancer and some chronic diseases such as cardiovascular diseases and neurodegenerative disorders. The most universal properties are related to their functions as antioxidants, signal transducer blockers or antiinflammatory agents. These effects are believed to be relevant to their therapeutic use, but this future potential of flavonoids and flavanoids needs to be substantiated through further experimental and clinical evidence.

Acknowledgements

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References

1. Cody V. et al. (Eds.). Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships. Alan Liss. Inc.: New York 1986.

2. Ho, C.T. et al.(Eds.). Phenolic Compounds in Food and Their Effects on Health I. Analysis, Occurrence and Chemistry. ACS Symposium Series 506, American Chemical Society: Washington, DC 1992.

3. Das, D.K. *Naturally occurring flavonoids: Structure, chemistry and high-performance liquid chromatography methods for separation and characterization.* Methods Enzymol 1994, 234: 410-20.

4. Huang, M.T. et al. (Eds.). Phenolic Compounds in Food and Their Effects on Health II. Antioxidant and Cancer Chemoprevention. ACS Symposium Series 507, American Chemical Society: Washington, DC 1992.

5. Block, G., Langseth, L. *Antioxidant vitamins and disease prevention.* Food Technol 1994, July: 80-4.

6. Block, G. *A role for antioxidants in reducing cancer risk.* Nutr Rev 1992, 50: 207-13.

7. Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., Kromhout, D. *Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly Study.* Lancet 1993, 342: 1007-11.

8. Ho, C.T. *Antioxidant properties of plant flavanoids.* In: Food Factors for Cancer Prevention. Ohigashi, H. et al. (Eds.). Springer-Verlag: Tokyo 1997, 593-7.

9. Samuelsson, G. (Ed.) Drugs of Natural Origin. Textbook of Pharmacognosy. Swedish Pharmaceutical Press 1993.

10. Kaul, N., Siveski-Iliskovic, N., Hill, M., Slezak, J., Singal, P.K. *Free radicals and the heart.* J Pharmacol Toxicol Methods 1993, 30: 55-67.

11. Bors, W., Heller, W., Michel, C., Saran, M. *Flavonoids as antioxidants: Determination of radical-scavenging efficiencies.* Methods Enzymol 1990, 186: 343-55.

12. van Acker, S.A., van den Berg, D.J., Tromp, M.N. et al. *Structural aspects of antioxidant activity of flavonoids.* Free Radic Biol Med 1996, 20: 331-42.

13. Halliwell, B. *Free radicals, antioxidants and human disease: Curiosity, cause or consequence?* Lancet 1994, 344: 721-4.

14. Cao, G., Sofic, E., Prior, R.L. *Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships.* Free Radic Biol Med 1997, 22: 749-60.

15. Yoshida, Y., Tsuchiya, J. Niki, E. *Interaction of α-tocopherol with copper and its effect on lipid peroxidation.* Biochem Biophys Acta 1994, 1200: 85-92.

16. Iwatsuki, M., Niki, E., Stone, D., Darley-Usmar, V.M. *α-Tocopherol mediated peroxidation in the copper(II) and met myoglobin induced oxidation of human low density lipoprotein: The influence of lipid hydroperoxides.* FEBS Lett 1995, 360: 271-6.

17. Sato, K., Akaike, T., Kohno, M., Naeda, H. *Hydroxyl radical production by H_2O_2 plus Cu, Zn-superoxide dismutase reflects the activity of free copper released from the oxidatively damaged enzyme.* J Biol Chem 1992, 267: 25371-7.

18. Rice-Evans, C.A., Miller, N.J., Paganga, G. *Structure-antioxidant activity relationships of flavonoids and phenolic acids.* Free Radic Biol Med 1996, 20: 933-56.

19. Morel, L., Lescoat, G., Cogrel, P. et al. *Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures.* Biochem Pharmacol 1993, 45: 13-9.

20. Morel, L., Lescoat, G., Cillard, P., Cillard, J. *Role of flavonoids and iron chelation in antioxidant action.* Methods Enzymol 1994, 243: 437-43.

21. Parmar, N.S., Ghosh, M.N. *Flavonoids and bioflavonoids.* In: Proceedings of the 6th Hungarian Bioflavonoids Symposium. Farkas L. et al.(Eds.). Elsevier: Amsterdam 1981, 513-6.

22. Hla, T., Neilson, K. *Human cyclooxygenase-2 cDNA.* Proc Natl Acad Sci USA 1992, 89: 7384-8.

23. Vane, J.R., Bakhle, Y.S., Bolting, R.M. *Cyclooxygenase 1 and 2.* Annu Rev Pharmacol Toxicol 1998, 38: 97-120.

24. Liang, Y.C., Huang, Y.T., Tsai, S.H., Lin-Shiau, S.Y., Chen, C.F., Lin, J.K. *Inhibition of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages.* Carcinogenesis 1999, 20: 1945-52.

25. Hecker, M., Preiss, C., Klemm, P., Busse, R. *Inhibition by antioxidants of nitric oxide synthase expression in murine macrophages: Role of nuclear factor-κB and interferon regulatory factor 1.* Br J Pharmacol 1996, 118: 2178-84.

26. Wadsworth, T.L., Koop, D.R. *Effects of the wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages.* Biochem Pharmacol 1999, 57: 941-9.

27. Yunet, H.Y., Dawson, V.L. Dawson, T.M. *Neurobiology of nitric oxide.* Crit Rev Neurobiol 1996, 10: 291-316.

28. Szabo, C. *Alteration in nitric oxide production in various forms of circulatory shock.* New Horiz 1995, 3: 2-32.

29. Neurath, M.F., Becker, C., Barbulescu, K. *Role of NFκB in immune and inflammatory responses in the gut.* Gut 1998, 43: 856-60.

30. Kujubu, D.A., Fletcher, B.S., Varnum, B.C., Lim, R.W., Herschman, H.R. *TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue.* J Biol Chem 1991, 266: 12866-72.

31. Lowenstein, C.J., Alley, E.W., Raval, P. et al. *Macrophage nitric oxide synthase gene: Two upstream regions mediate induction by interferon γ and lipopolysaccharide.* Proc Natl Acad Sci USA 1993, 90: 9730-4.

32. Imber, T., Rupec, R.A., Livolsi, A.C. et al. *Tyrosine phosphorylation of IκBα-activates NFκB without proteolytic degradation of IκBα.* Cell 1996, 86: 787-98.

33. Tsai, S.H., Lin-Shiau, S.Y., Lin, J.K. *Suppression of nitric oxide synthase and the down-regulation of the activation of NFκB in macrophages by resveratrol.* Br J Pharmacol 1999, 126: 673-80.

34. Hertog, M.G.L., Hallman, P.C., Katan, M.B., Kromhout, D. *Intake of potentially anticarcinogenic flavonoids and their determination in The Netherlands.* Nutr Cancer 1993, 20: 21-9.

35. Wei, N., Tye, L., Bresnick, E., Birt, D.F. *Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice.* Cancer Res 1990, 50: 499-502.

36. Verma, A.K., Johnson, J.A., Gould, M.N., Tanner, M.A. *Inhibition of DMBA and NMU-induced mammary cancer by dietary flavonol quercetin.* Cancer Res 1988, 48: 5754-8.

37. Lin, J.K., Chen, Y.C., Huang, Y.T., Lin-Shiau, S.Y. *Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin.* J Cell Biochem 1997, Suppl. 28/29: 39-48.

38. Huang, Y.T., Kuo, M.L., Liu, J.Y., Lin, J.K. *Inhibition of protein kinase C and proto-oncogene expression in NIH3T3 cells by apigenin.* Eur J Cancer 1996, 32A: 146-51.

39. Wang, H., Murphy, P.A. *Isoflavone content in commercial soybean foods.* J Agric Food Chem 1994, 42: 1666-73.

40. Barnes, S., Grubbs, C., Setchell, K.D.R., Carlson, J. *Soybeans inhibit mammary tumors in models of breast cancer.* Prog Clin Biol Res 1990, 347: 239-53.

41. Hawrylewicz, E.J., Huang, H.H., Blair, W.H. *Dietary soybean isolate and methionine supplementation affect mammary tumor progression in rats.* J Nutr 1991, 121: 1693-8.

42. Fritz, W.A., Coward, L., Wang, J., Lamartiniere, C.A. *Dietary genistein: Perinatal mammary cancer prevention, bioavailability and toxicity testing in rat.* Carcinogenesis 1998, 19: 2151-8.

43. Folman, Y., Pope, G.S. *The interaction in the immature mouse of potent estrogens with coumestrol, genistein and other utero-vaginotrophic compounds of low potency.* J Endocrinol 1966, 34: 215-25.

44. Akiyama, T., Ishida, J., Nakayama, O. et al. *Genistein, a specific inhibitor of tyrosine specific protein kinase.* J Biol Chem 1987, 262: 5592-5.

45. Weisburger, J.H., Hara, Y., Dolan, L. et al. *Tea polyphenols as inhibitors of mutagenicity of major classes of carcinogens.* Mutat Res 1996, 371: 57-63.

46. Hour, T.C., Liang, Y.C., Chu, I.S., Lin, J.K. *Inhibition of eleven mutagens by various tea extracts, (–)-epigallocatechin-3-gallate, gallic acid and caffeine.* Food Chem Toxicol 1999, 37: 569-79.

47. Apostolides, Z., Balentine, D.A., Harbowy, M.E., Weisburger, J.H. *Inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) mutagenicity by black and green tea extracts and polyphenols.* Mutat Res 1996, 359: 159-63.

48. Hernaez, J.F., Xu, M., Dashwood, R.H. *Antimutagenic activity of tea towards 2-hydroxyamino-3-methylimidazo[4,5-f]quinoline: Effect of tea concentration and brew time on electrophile scavenging.* Mutat Res 1998, 402: 299-306.

49. Chen, C.W., Ho, C.T. *Antioxidant properties of polyphenols extracted from green and black teas.* J Food Lipids 1994, 2: 35-46.

50. Greenberg, L.N., Newmark, H., Kitrosky, N. et al. *Protective effects of tea polyphenols against oxidative damage to red blood cells.* Biochem Pharmacol 1997, 54: 973-8.

51. Halder, J., Bhaduri, A.N. *Protective role of black tea against oxidative damage of human red blood cells.* Biochem Biophys Res Commun 1998, 244: 903-7.

52. Khan, S.G., Mohan, R.R., Agarwal, R., Mukhtar, H. *Enhancement of antioxidant and phase II enzyme by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: Possible role in cancer chemoprevention.* Cancer Res 1992, 52: 4050-2.

53. Lee, S.F., Liang, Y.C., Lin, J.K. *Inhibition of 1,2,4-benzene triol-generated reactive oxygen species and induction of phase II enzymes by green tea polyphenols.* Chem Biol Interact 1995, 98: 283-301.

54. Lin, Y.L., Cheng, C.Y., Lin, Y.P., Lau, Y.W., Juan, I.M., Lin, J.K. *Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, glutathione S-transferase in rats.* J Agric Food Chem 1998, 46: 1893-9.

55. Lin, J.K., Chen, P.C., Ho, C.T., Lin-Shiau, S.Y. *Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3,3'-digallate, (–)-epigallocatechin-3-gallate and propyl gallate.* J Agric Food Chem 2000, 48: 2736-43.

56. Katiyar, S.K., Mukhtar, H. *Inhibition of phorbol ester tumor promoter TPA-caused inflammatory response in SENCAR mouse skin by black tea polyphenols.* Carcinogenesis 1997, 18: 1911-6.

57. Moncada, S.R., Palmer, M.J., Higgs, D.A. *Nitric oxide: Physiology, pathophysiology and pharmacology.* Pharmacol Rev 1992, 43: 109-42.

58. Lin, Y.L., Lin, J.K. *(–)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating LPS-induced activity of transcription factor nuclear factor-κB.* Mol Pharmacol 1997, 52: 465-72.

59. Lin, Y.L., Tsai, S.H., Lin-Shiau, S.Y., Ho, C.T., Lin, J.K. *Theaflavin-3,3'-digallate from black tea blocks the nitric oxide synthase by down-regulating the activation of NFκB in macrophage.* Eur J Pharmacol 1999, 367: 397-88.

60. Huang, M.T., Wood, A.W., Conney, A.H. et al. *Inhibition of the mutagenicity of bay-region diol epoxide of polyphenolic aromatic hydrocarbons by phenolic plant flavonoids.* Carcinogenesis 1983, 4: 1631-7.

61. Katiyar, S.K., Mutkhtar, H. *Tea antioxidants in cancer chemoprevention.* J Cell Biochem 1997, Suppl. 27: 59-67.

62. Lin, J.K., Liang, Y.C. *Cancer chemoprevention by tea polyphenols.* Proc Natl Sci Council ROC (B) 2000, 24: 1-13.

63. Taniguchi, S., Fujiki, H., Kobayashi, H. et al. *Effect of(–)-epigallocatechin-3-gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines.* Cancer Lett 1992, 65: 51-4.

64. Kelloff, G.J., Boone, C.W., Crowell, J.A. et al. *New agents for cancer chemoprevention.* J Cell Biochem 1996, Suppl. 26: 1-28.

65. Lin, J.K., Liang, Y.C., Lin, Y.L., Lin-Shiau, S.Y. *Modulation of mitotic signal transduction by curcumin and tea polyphenols and their implication for cancer chemoprevention.* In: Functional Foods for Disease Protection. Shibamoto, T. et al (Eds.). Symposium Series 701, American Chemical Society: Washington, DC 1998, 225-38.

66. Lin, J.K., Liang, Y.C., Lin-Shiau, S.Y. *Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade.* Biochem Pharmacol 1999, 58: 911-5.