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REVIEW

Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease

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Abstract Chronic inflammation is being shown to be increasingly involved in the onset and development of several pathological disturbances such as arteriosclerosis, obesity, diabetes, neurodegenerative diseases and even cancer. Treatment for chronic inflammatory disorders has not been solved, and there is an urgent need to find new and safe anti-inflammatory compounds. Flavonoids belong to a group of natural substances occurring normally in the diet that exhibit a variety of beneficial effects on health. The anti-inflammatory properties of flavonoids have been studied recently, in order to establish and characterize their potential utility as therapeutic agents in the treatment of inflammatory diseases. Several mechanisms of action have been proposed to explain in vivo flavonoid anti-inflammatory actions, such as antioxidant activity, inhibition of eicosanoid generating enzymes or the modulation of the production of proinflammatory molecules. Recent studies have also shown that some flavonoids are modulators of proinflammatory gene expression, thus leading to the attenuation of the inflammatory response. However, much work remains to be done in order to achieve definitive conclusions about their potential usefulness. This review summarizes the known mechanisms involved in the anti-inflammatory activity of flavonoids and the implications of these effects on the protection against cancer and cardiovascular disease.

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Introduction

Inflammation is an orchestrated biological process, induced by microbial infection or tissue injury. A major trigger of inflammation is the recognition of microbes by specific receptors of the innate immune system, which play a crucial role in the induction of early signals initiating and establishing the inflammatory setting [1]. A main function of inflammation is to resolve infection and to repair the damage in order to achieve homeostasis equilibrium. Thus, the ideal inflammatory response is rapid and destructive, yet specific and self-limiting [2]. The importance of this balance is demonstrated by findings in certain chronic infectious or inflammatory disorders, that the inflammatory response causes more damage to the host than the microbe.

Inflammation and the immune system are intimately tied. Indeed, an over activation of innate immune response can cause chronic infection or chronic inflammation due to an inefficient regulation or resolution of the inflammatory response [3].

Although steroidal anti-inflammatory drugs and NSAIDs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders while such compounds are accompanied by unexpected side effects. Therefore, there is an urgent need to find safer anti-inflammatory compounds [4]. Traditional medicine has used extracts of different plants for the treatment of a wide variety of disorders including acute and chronic inflammation. Among the active constituents of these extracts, flavonoids are a family of substances whose members have many interesting biological properties

including anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic activities [5–7].

Among these biological activities, the anti-inflammatory capacity of flavonoids has long been utilized in Chinese medicine by applying crude plant extracts. Many investigations have shown that a variety of flavonoid molecules exhibit anti-inflammatory activity both, in vitro and in various animal models of inflammation [8, 9].

In addition, inflammation is increasingly found to be involved in the development of several chronic diseases such as arteriosclerosis, obesity, diabetes, neurodegenerative diseases and even cancer. Among them, cardiovascular diseases and cancer are main causes of mortality in many countries. Numerous epidemiological studies indicate that an increase in the consumption of flavonoid-rich fruits and vegetables is associated with a decrease in the incidence of cardiovascular disease and different types of cancer [10–15]. This protective effect has been attributed in part to anti-inflammatory properties of flavonoids [16]. Thus, it may be valuable to study the anti-inflammatory activity of flavonoids, not only in order to establish anti-inflammatory mechanisms, but also for developing a new class of safe anti-inflammatory agents, which may be useful in the treatment of these kind of diseases [17].

This document reviews the anti-inflammatory properties of flavonoids with special emphasis on the various mechanisms potentially implicated. We also summarize the central role that inflammation plays in the onset and progression of two of the most important diseases of the world: cancer and cardiovascular disease. The possible effects of flavonoids in the prevention and treatment of such diseases are also reviewed, on the basis of their anti-inflammatory activity.

Flavonoids and inflammation

Flavonoids are a polyphenols subclass which are widely distributed in the plant kingdom, and are characterized by two or more aromatics rings, each bearing at least one aromatic hydroxyl and connected with a heterocyclic pyran [18]. Flavonoids are categorized into different subtypes based on the connection of an aromatic ring to the heterocyclic ring as well as the oxidation state and functional groups of the heterocyclic ring. Flavonoids are found in fruits, vegetables, legumes, herbs, spices, stems, flowers as well as tea and red wine. They are prominent components of citrus fruits and other food sources and are in many countries regularly consumed in a healthy diet. Table 1 shows the subclasses of flavonoids and the names of prominent food flavonoids and typical food sources [18].

Many investigations have repeatedly proven that different flavonoid molecules exhibit anti-inflammatory functions. Thus, the anti-inflammatory activities of flavonols (quercetin, rutin and morin) and flavanones (hesperetin and hesperidin) were investigated in acute and chronic inflammation animal models [8]. Rutin was only effective in the chronic process, principally in adjuvant arthritis. On neurogenic inflammation induced by xylene, only the flavanones were effective. Besides, these compounds were the most effective on the subchronic inflammatory process. The most important compound in reducing paw edema induced by carrageenan was quercetin [8]. Paradkar et al. [19] demonstrated that an isoflavone-containing diet with daidzin, glycitin, genistein and their glucosides, can modulate the inflammatory reaction in the intestine and liver of mice after LPS injection. These in vivo findings were consistent with the anti-inflammatory effect of genistein found in cell studies using human intestinal CACO-2 cells.

Among a great variety of natural flavonoids, one of the most studied in different models of inflammation has been the genistein (an isoflavone). The effect of this compound has been evaluated on a guinea pig model of asthma [20]. In this model of airway inflammatory disease, genistein markedly attenuates ovalbumin-induced bronchoconstriction, pulmonary eosinophilia and airway hyperresponsiveness. This anti-inflammatory effect may be mediated by the inhibition of the tyrosine kinase signaling cascade [20]. Intraperitoneally injected genistein was shown to protect rats from the endotoxin-induced organ failure [21], and later treatment with genistein reduced the degree of inflammation and joint destruction in collagen induced arthritic mice. This therapeutic effect was mediated by a modulation of granulocytes, monocytes and lymphocytes [22]. Other flavonoids have been shown to be effective in preventing adjuvant arthritis in the rat. Daily intraperitoneal administration of rutin, quercetin and hesperidin, inhibited both acute and chronic phases in this experimental model of inflammation, with rutin being the most active compound in the chronic phase [23].

The anti-inflammatory activity of flavonoids has been also investigated in in vitro models, where a number of studies have been conducted to elucidate the mechanisms of action.

Anti-inflammatory mechanisms of flavonoids

Several mechanisms explaining the anti-inflammatory activity of flavonoids have been described, including (a) antioxidative and radical scavenging activities, (b) regulation of cellular activities of inflammation-related cells, (c) modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase,

Table 1 Subclasses and prominent food flavonoids and typical food sources

Flavonoid subclass	Food flavonoid	Food source
Flavanols	Catechin, gallocatechin, epicatechin	Teas, red grapes and red wines
Flavanones	Naringenin, hesperetin, eriodictyol	Citrus foods
Flavones	Apigenin, luteolin	Green leafy spices
Isoflavones	Daidzein, genistein, glycitein, biochanin A	Soybeans, soy foods, and legumes
Flavonols	Kaempferol, myricetin, quercetin, isorhamnetin	Nearly ubiquitous in foods
Anthocyanidins	Cyanidin, delphinidin, pelargonidin	Red, purple and blue berries

lipoxygenase) and nitric oxide synthase, (d) modulation of the production of other proinflammatory molecules, (e) modulation of proinflammatory gene expression.

Flavonoids as antioxidants

Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous factors [24]. The increased production of reactive oxygen species accompany most forms of tissue injury, which have been implicated in a multitude of disease states ranging from inflammatory injury to myocardial infarction and cancer [25]. The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood, but some of the detrimental effects in biological systems include peroxidation of membrane lipids, oxidative damage to nucleic acids or carbohydrates and the oxidation of sulfhydryl and other susceptible groups in proteins [26, 27]. In addition, free radicals can attract various inflammatory mediators contributing to a generalized inflammatory response and tissue damage. Indeed, flavonoids are powerful in vitro antioxidants, being able to scavenge a wide range of free radical species, as well as to inhibit their formation.

Effect on ROS production by phagocytic cells

Phagocytosis is an important physiological process accompanied by the production of superoxide anions. While ROS generated by phagocytes play an important physiological function, they can also cause cellular damage. The highly reactive oxygen species, along with other mediators elaborated by neutrophils and macrophages, can promote inflammation and cause tissue damage [28, 29]. Several flavonoids have been shown to be effective inhibitors of ROS production by activating human neutrophils [30–32].

Radical scavenging

Flavonoids are scavengers of a wide variety of reactive oxygen, nitrogen, and chlorine species such as superoxide, hydroxyl radical, peroxyl radicals, hypochlorous acid and peroxynitrous acid, since they are oxidized by radicals, resulting in a more stable, less reactive radical [33]. Selected flavonoids can directly scavenge superoxides [34], whereas other flavonoids such as genistein and daidzein can scavenge the highly reactive oxygen-derived radical peroxynitrite [35]. Epicatechin and rutin have a powerful hydroxyl radical (OH·) scavenging effect, about 100–300 times higher than mannitol, a typical OH· scavenger, and also inhibit the superoxide anion (O_2^-) generation in the hypoxanthine-xanthine oxidase system [36]. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro, [37] protecting the LDL particles. Such effect may have preventive actions against atherosclerosis.

During inflammation, high concentrations of nitric oxide produced by inducible nitric oxide synthase in macrophages can result in oxidative damage. In such circumstances, activated macrophages greatly increase the simultaneous production of both nitric oxide and superoxide anions. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite that can directly oxidize LDL, resulting in irreversible damage to the cell membrane [34]. When flavonoids are used as antioxidants, free radicals are scavenged and, therefore, can no longer react with nitric oxide, resulting in less cellular damage [38]. Also, nitric oxide can be viewed as a radical itself, and it has been reported that nitric oxide molecules are directly scavenged by flavonoids [39]. The soybean isoflavones genistein and daidzein increase LDL resistance to peroxynitrite-mediated oxidation, in vitro, in a concentration-dependent fashion [35]. In vivo experiments have demonstrated that oral administration of isoflavones and extracts from soy-based products decrease serum nitrite, nitrate and nitrotyrosine levels in LPS-induced rats [40]. Thus, isoflavone supplementation may inhibit reactive nitrogen species-induced oxidation, helping to provide a protective effect against cardiovascular and chronic inflammatory diseases.

Inhibition of pro-oxidant enzymes

Stimulation of inflammatory cells such as macrophages by bacterial endotoxins or inflammatory cytokines results in

increased expression of inducible nitric oxide synthase (iNOS) and subsequent production of large amount of nitric oxide that is able to produce oxidative injury. Flavonoids and other natural polyphenols can inhibit lipopolysaccharide-induced iNOS gene expression and iNOS activity in cultured macrophages [41, 42] by reducing the nitric oxide production and, subsequently, oxidative damage.

Lipoxygenases and cyclooxygenases are capable of co-oxidizing molecules other than their regular substrates, with the potential for increasing oxidative lesion in some tissues. Some flavonoids and other plant polyphenols have the ability to inhibit cyclooxygenase (COX-2) and lipoxygenase [43–45].

The xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissues. During ischemic conditions, xanthine dehydrogenase changes to xanthine oxidase that is a source of oxygen free radicals. Some flavonoids inhibit xanthine oxidase activity, resulting in decreased oxidative injury [46].

Indeed, a variety of oxidants, free radicals and aldehydes are implicated in the pathogenesis of chronic inflammatory diseases, since polyphenolic components from dietary plants may increase the endogenous antioxidant potential and, thus, modulate cellular redox state. These compounds may be an alternative for the treatment of chronic inflammatory diseases.

Modulation of inflammatory related cell functions

The immune system is integrated by a highly complex regulated group of cells that may interact in a cell–cell manner and may also respond to intercellular messages including hormones, cytokines and autacoids. The immune response can be modified by diet, pharmacological agents, environmental pollutants, and naturally occurring food chemicals such as vitamins and flavonoids [47–49]. Some flavonoids display a remarkable array of biochemical and pharmacological actions that affect the function of immune and inflammatory cells such as T cells, B cells, macrophages, neutrophils, mast cells, or basophils [50].

Several flavonoids specifically affect enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases. These enzymes are involved in signaling transduction and cell activation processes such as T cell proliferation [51, 52], B lymphocyte activation [53] or cytokine production by stimulated monocytes [54]. Genistein, an isoflavone, has been demonstrated as a specific inhibitor for tyrosine protein kinase [55]. This activity may be involved in some of its anti-inflammatory effects, while T cell proliferation is accompanied by phosphorylation of tyrosine of particular T cell proteins. Trevillyan et al. [56] showed that the inhibition of the enzymatic activity of the T cell specific protein kinase p56lck by genistein correlated with a reduced IL-2 secretion and IL-2R expression in T cells stimulated with PHA/PMA. Also, PTK activation is required for LPS induction and release of cytokines such as TNF-α, IL-6 and IL-1β from human blood monocytes [54, 57]. In in vitro studies with human peripheral mononuclear cells, genistein at a non-cytotoxic concentration, inhibited cell proliferation, and IL-2 and LTB4 production from stimulated cultures [58]. Geng and coworkers [54] demonstrated that a tenfold increase in mRNA of IL-1β, IL-6 and TNF-α produced by LPS-stimulated monocytes was blocked by genistein, which also reduced the LPS-induced activation of nuclear factor κB (NF-κB), a transcription factor involved in the expression of cytokine genes, illustrating a potentially very important flavonoid–gene interaction. Other flavones such as apigenin, chrysin or luteolin and flavonols such as kaempferol and quercetin showed remarkable antiproliferative effects on M-CSF-activated macrophages, which may be related with their role as tyrosine kinase inhibitors [59].

Flavonoids also exhibit an effect on secretory processes of inflammatory cells. Thus, Bennett et al. [60] have shown that several flavonoids were capable of inhibiting stimulated rabbit neutrophil lysosomal enzyme release. In other studies, quercetin impaired secretion of lysosomal enzyme from human polymorphonuclear leukocytes induced by concanavalin A [61]. Quercetin also inhibited human neutrophil degranulation as well as catalytic activity of the released elastase [62]. Oral administration of rutin reduced in a dose-dependent manner the polymorphonuclear neutrophils chemotaxis to FMLP in a model of rat paw oedema [63]. Several flavonoids such as luteolin, kaempferol, apigenin, or quercetin have been reported as potent inhibitors of β-glucuronidase and lysozyme release from neutrophils [64]. These flavonoids significantly inhibited arachidonic acid release from membranes, an effect that was correlated with degranulation [64].

Modulation of proinflammatory enzyme activities

Many investigations have shown that different flavonoid molecules modulate the activity of arachidonic acid (AA) metabolizing enzymes such as phospholipase A_2(PLA_2) [65, 66], cyclooxygenase (COX) and lipoxygenase (LOX) [67] and the nitric oxide (NO) producing enzyme, nitric oxide synthase (NOS) [68, 69]. The inhibition of these enzymes reduces the production of AA, prostaglandins, leucotrienes, and NO, which are crucial mediators of inflammation. Thus, the inhibition of these enzymes by flavonoids may be one of the most important mechanisms of their anti-inflammatory activity.

Arachidonic acid related enzymes

Arachidonic acid release is a starting point for a general inflammatory response. Arachidonic acid is released from membrane phospholipids in cells by the action of PLA_2, and metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) pathways to prostaglandins, vasoactive leukotrienes LTC_4, LTD_4, LTE_4, as well as to the potent chemoattractant LTB_4 [50]. Selected phenolic compounds such as flavonols and polyphenols were found to inhibit these enzymes, reducing the release and metabolism of arachidonic acid and thus, diminishing the formation of inflammatory mediators.

The first described flavonoid inhibitor of PLA_2 was quercetin, which inhibited PLA_2 from human neutrophils [70]. Later, several studies have repeatedly reported that quercetin and other flavonoids inhibit different isoforms of PLA_2 from different sources [65, 66, 71, 72].

Cyclooxygenase (COX) produces prostaglandins (PG) and thromboxanes from AA. The enzyme exists in two different isoforms COX-1 and COX-2. Thus, COX-1 is a constitutive enzyme existing in almost every cell type, while COX-2 is an inducible enzyme that produces large quantities of PG, and is highly expressed in the inflammation related cells when they are stimulated with proinflammatory cytokines and/or bacterial lipopolysaccharide [73, 74]. Lipoxygenases (LOXs) are responsible for generating hydroxy acids and leukotrienes from AA. Among the different isoforms of LOX, 5- and 12-LOX are involved in allergic and inflammatory disorders, 5-LOX produces 5-HETE and LTs, which are potent chemoattractants, 12-LOX synthesizes 12-HETE, which aggregates platelets and induces inflammatory response [75].

Some flavonoids such as luteolin, galangin or morin were for the first time described as inhibitors of COX [76]. From human thrombin aggregated platelets, certain flavonoids were identified as COX/LOX inhibitors, and this antagonistic activity was related with the structural characteristics of the different molecules: flavone derivatives such as flavone, apigenin, and chrysin inhibited platelet aggregation by depressing the COX pathway, while flavonol-related compounds such as myricetin and quercetin inhibited primarily LOX activity [77]. Flavonoids inhibiting COX-2 activity has been rarely reported, Chi et al. [67] compared the effect of different flavonoid derivates on COX-1, COX-2, 5-LOX and 12-LOX activity. Among the studied molecules some prenylated flavonoids moderately inhibited COX-2, but with low selectivity over COX-1. Wogonin, a plant derived flavone was found to inhibit COX-2 activity as well as COX-2 expression in LPS induced macrophages [78, 79]. This compound did not significantly inhibit COX-1 and 12-LOX from human platelet homogenates [80]. The inhibitory effect of wogonin on COX-2 activity may be a selective effect, since this compound inhibits PGE_2 production, but not LTB_4 from IL-1β induced gingival fibroblasts [81].

The inhibition of 5-LOX from human polymorphonuclear cells by isoflavones has been investigated [82]. Thus, it has been shown that isoflavones act as redox inhibitors that can regulate lipoxygenase activity by preventing activation of resting form (ferrous state) to its reactive state (ferric) and simultaneously can convert the active form of lipoxygenase to its resting state. Among the molecules studied, genistein was a more potent inhibitor of LOX than daidzein, while glycosylated forms were as potent as their aglycones [82].

The LOX pathway generates leukotrienes. When COX-2 is blocked, the LOX pathway still produces the potent mediators of inflammation. Dual inhibition of COX/LOX has been suggested to be a relevant approach in the development of new anti-inflammatory treatments [4]. Some natural polyphenols such as curcumin are inhibitors of both COX and LOX. These compounds can modulate arachidonic acid metabolism at different stages, by inhibiting phosphorylation of cPLA, inhibiting COX-2 protein expression and catalytic activity, and inhibiting 5-LOX activity [83].

NO synthase

NO, a ubiquitous cellular mediator of physiological and pathological processes, is produced by a family of enzymes, including endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). The latter type is an inducible enzyme that is highly activated by inflammatory stimuli (LPS and inflammatory cytokines) in certain cells such as macrophages [84]. Indeed, iNOS is responsible for the overproduction of NO during inflammation. Thus, compounds that are able to reduce NO production by iNOS without affecting eNOS or nNOS may be desirable as anti-inflammatory agents. Certain flavonoids have been shown to inhibit NO production from macrophage or macrophage-like cells activated with inflammatory stimuli [85–89].

In this context, it has been reported that the high affinity of polyphenols for proteins and a possible subsequent conformational change of enzyme might be associated with the inhibitory effect by flavonoids on iNOS enzyme activity [68]. However, only a few studies have demonstrated a direct effect of flavonoids on enzyme activity. Cheon et al. (2000) studied the effects of some prenylated flavonoids and biflavonoids on LPS-induced nitric oxide production from RAW 264.7 cell line. These investigators found that such compounds inhibited the production of nitric oxide, this effect being mediated by the suppression of iNOS enzyme induction, but not by direct inhibition of

iNOS activity. The only reported exception was echinoisoflavone, which inhibited iNOS enzyme activity and suppressed iNOS enzyme induction [69]. Studies with soy isoflavones genistein, daidzein and glycitein have revealed that all of them are able to dose-dependently suppress NO production in LPS-activated murine macrophages by three different mechanisms: scavenging of NO radicals, inhibition of iNOS enzyme activity and inhibition of iNOS gene expression [89]. In contrast, other mechanistic studies have shown that the inhibitory activity of flavonoids was not due to a direct effect on enzyme activity, but was through a reduction of iNOS enzyme expression [87, 90].

Modulation of the production of other proinflammatory molecules

In addition to COX-2 and iNOX, several cytokines are deeply associated with inflammatory diseases. In particular, tumor necrosis factor-α (TNF-α), IL-6 and IL-1β are prominent contributors to chronic inflammatory responses. Genistein was reported to inhibit IL-1β, IL-6 and TNF-α production in LPS-induced human blood monocytes [54].The inhibitory effect of genistein on IL-6 production has been shown in different settings: cultured human intestinal cells Caco-2 [19], osteoblast cells [91], human gastric epithelial cells [92], or macrophages [93]. Pretreatment of the macrophage cell line RAW 264.7 with luteolin, luteolin-7-glucoside, quercetin, and genistein inhibited both LPS-stimulated TNF-α and IL-6 release, whereas erodictyol and hesperetin only inhibited TNF-α release. Luteolin and quercetin were able to block TNF-α release by more than 80%.

The comparison of molecular structures from different flavonoids shows that the presence of a double bond at position C2–C3 of the C ring with oxo function at position 4, along with the presence of OH groups at positions 3′ and 4′ of the B ring, are required for optimal inhibition of LPS-stimulated TNF-α release [93]. Amoradicin, genistein, and silybin were shown to inhibit TNF-α production from LPS-treated RAW 264.7 cells [94]. Quercetin inhibited IL-1β, IL-6 and TNF-α production in LPS-stimulated RAW 264.7 cells [95]. Wogonin reduces the in vitro TNF-α production in LPS stimulated RAW cells and decreases the in vivo level of circulating TNF-α in mice administrated D-galactosamine and LPS [96].

Modulation of proinflammatory gene expression

In recent years, several lines of evidence have supported the idea that certain flavonoids are modulators of proinflammatory gene expression, thus leading to the attenuation of the inflammatory response. It is not known to what extent these proinflammatory gene expression changes contribute to the inflammatory response but is evident that flavonoids show anti-inflammatory activity, at least in part, by affecting mRNA levels. The mechanisms by which flavonoids block proinflammatory gene expression are currently being investigated, but pioneer studies suggest an effect on transcriptional activity suppression in response to inflammatory stimuli [97].

COX-2 selective inhibitors are claimed to show anti-inflammatory activity and are continuously being developed to obtain safer anti-inflammatory drugs. Flavonoids inhibiting COX-2 activity are rarely reported, but some studies have demonstrated an effect on suppression of COX-2 expression. Thus, apigenin, genistein, and kaempferol strongly inhibited COX-2 induction in LPS-stimulated macrophages [85]. Other experiments using the gene-reporter assay to express COX-2 showed that some flavones and flavonols were active suppressors, but epigallocatechin-3-gallate, catechin, and myricetin were not [98]. In a recent work, Hooshmand et al. [99] have found that genistein selectively decreases the production of LPS-induced COX-2 protein level in chondrocytes without affecting COX-1. Luteolin decreases protein and mRNA levels of the proinflammatory iNOS and COX-2 in LPS-stimulated macrophages [100].

Several studies have shown that some flavonoids inhibit NO production in response to inflammatory stimuli [85–89]. Hämäläinen et al. compared the effects of a series of compounds on NO production. The flavonoid classes containing the most effective compounds were isoflavones and flavonols. They identified eight compounds as being able to inhibit LPS-induced iNOS expression: flavones, daidzein, genistein, isorhamnetin, kaempferol, quercetin, naringenin and pelargonidin [101].

Mechanisms modulating gene expression

Cellular mechanisms of flavonoids modulating gene expression have been actively studied. The most prominent keys of cellular regulation affected by flavonoids are the various protein kinases involved in signal transduction including protein kinase C (PKC) and mitogen-activated protein kinase (MAPK). Through the inhibition of these enzymes, DNA-binding capacity of transcription factors such as NF-κB or activator protein-1 (AP-1) is regulated, and the expression rate of the gene target is controlled [17].

Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine kinases, which connect inflammatory and other extracellular signals to intracellular responses, such as gene expression [102]. The three better characterized MAPKs are extracellular signal-regulated kinase 1 and 2 (Erk1/2), p38, and c-Jun N-terminal kinase (JNK) [103]. P38-MAPK positively regulates a number of cytokine genes in vitro including TNF-α, IL-6 and iNOS [104, 105].

Cytokine production (TNF-α, IL-6, IL-10 and IL-1R antagonist) is strongly inhibited by the administration of a p38 MAPK inhibitor in vivo, during human endotoxemia [106]. In human chondrocytes, inhibition of JNK, p38, and Erk1/2 MAP kinases downregulates IL-1-induced COX-2 expression and PGE_2 production [107]. Inhibition of MAPKs is likely to result in a suppression of inflammatory mediators and these kinases may be a target for anti-inflammatory approaches. Exposure of mammalian cells to LPS has been shown to activate MAPK signaling cascades [108]. Xagorari et al. [109] have shown that the exposure of RAW 264.7 macrophages to LPS caused phosphorylation of ERK1/2, p38, and JNK pathways, pretreatment of cells with luteolin abolished the LPS-induced stimulation of ERK1/2 and p38, but not JNK phosphorylation. The contribution of ERK1/2 and p38 pathways in stimulated TNF-α production in macrophages depends on the origin of macrophages and the nature of the stimulus [110–113]. By using specific inhibitors, these researchers demonstrated that only simultaneous inhibition of the two pathways resulted in drastic reduction of TNF-α release [109], which is in agreement with results obtained with alveolar macrophages, where the activation of both ERK and p38 is necessary for optimal TNF-α production [114]. Similar results have been obtained with quercetin, where pretreatment of LPS-stimulated RAW 264.7 cells with quercetin inhibited ERK and p38 activation, but not JNK activation [95].

Another control point of gene expression is the NF-κB transcriptional system, which is a major effector pathway involved in inflammation and innate immune responses [115]. Many genes that are implicated in the initiation of inflammatory responses are regulated at the level of transcription by NF-κB. Activation of this nuclear factor is regulated by its endogenous inhibitor IκB, which complexes and sequesters NF-κB in the cytoplasm. Following stimulation, the successive activation of various kinases leads to the phosphorylation and degradation of IκB and subsequent release of NF-κB, which then translocates to the nucleus and activates the transcription of multiple genes, including TNF-α, IL-6, IL-8, and other chemokines; MHC class II; ICAM-1; iNOS, and COX-2 [116]. Several flavonoids have been shown to downregulate the production of inflammatory mediators through the blockade of NF-κB pathway at different levels.

In this context, luteolin has shown potent anti-inflammatory properties by inhibiting LPS-induced pro-inflammatory molecule expression both in vitro [93, 117] and in vivo [118].The molecular mechanisms of luteolin-mediated immunomodulation have been extensively studied in different cellular lines. In murine macrophages RAW 264.7, luteolin inhibits gene expression and proinflammatory cytokine production by blocking protein tyrosine phosphorylation and NF-κB activation [93]. In intestinal epithelial and dendritic cells, luteolin blocks LPS-induced NF-κB signaling and proinflammatory gene expression through the inhibition of IKK activity [119]. It has been reported in mouse alveolar macrophages that luteolin inhibits LPS-induced inflammatory reactions by blocking the NF-κB and AP-1 activation pathways [100].

Hämäläinen et al. studied the effect of eight flavonoid compounds on the activation of inflammatory transcriptional factors NF-κB and STAT-1. All of them inhibited LPS-induced NF-κB activation, but only four of them: genistein, kaempferol, quercitin and daidzein also inhibited STAT-1 activation. Interestingly, the three most potent antagonists of iNOS expression and NO production (genistein, kaempferol and quercitin) inhibit both NF-κB and STAT-1 activations, whereas those flavonoids inhibiting only NF-κB had smaller effect on iNOS expression [101].

Flavonoids, inflammation and disease

Excessive inflammation is considered to be a critical factor in many human diseases, including cancer, cardiovascular diseases, obesity, type II diabetes, or inflammatory bowel disease [97]. The reported anti-inflammatory properties of natural products such as flavonoids may be a crucial factor in using these substances for the treatment of such diseases.

Flavonoids, inflammation and cancer

Cancer is a hyperproliferative disorder that involves morphological cellular transformation, dysregulation of apoptosis, uncontrolled cellular proliferation, invasion, angiogenesis, and metastasis [120]. Clinical and epidemiologic studies have suggested a strong association between chronic infection, inflammation, and cancer [121–124]. Several lines of evidence are consistent with the view that inflammation plays a role in malignant processes: chronic inflammation predispose to cancer, immune inflammatory cells and inflammatory mediators are found in cancer, deletion of inflammatory mediators inhibits development of experimental cancers, and long-term use of nonsteroidal anti-inflammatory agents reduces the risk of some tumors [125]. These observations suggest that chronic inflammation is involved in tumor initiation, promotion and progression [121]. Recent data from mouse models of human cancer have established that inflammation, which orchestrates the tumor microenvironment, is a critical component of tumor evolution [126, 127]. Moreover, excessively and chronically produced proinflammatory mediators are thought to contribute to tumor promotion and progression [121, 126].

Chronically activated immune cells promote cancer development via direct and indirect mechanisms. Multiple

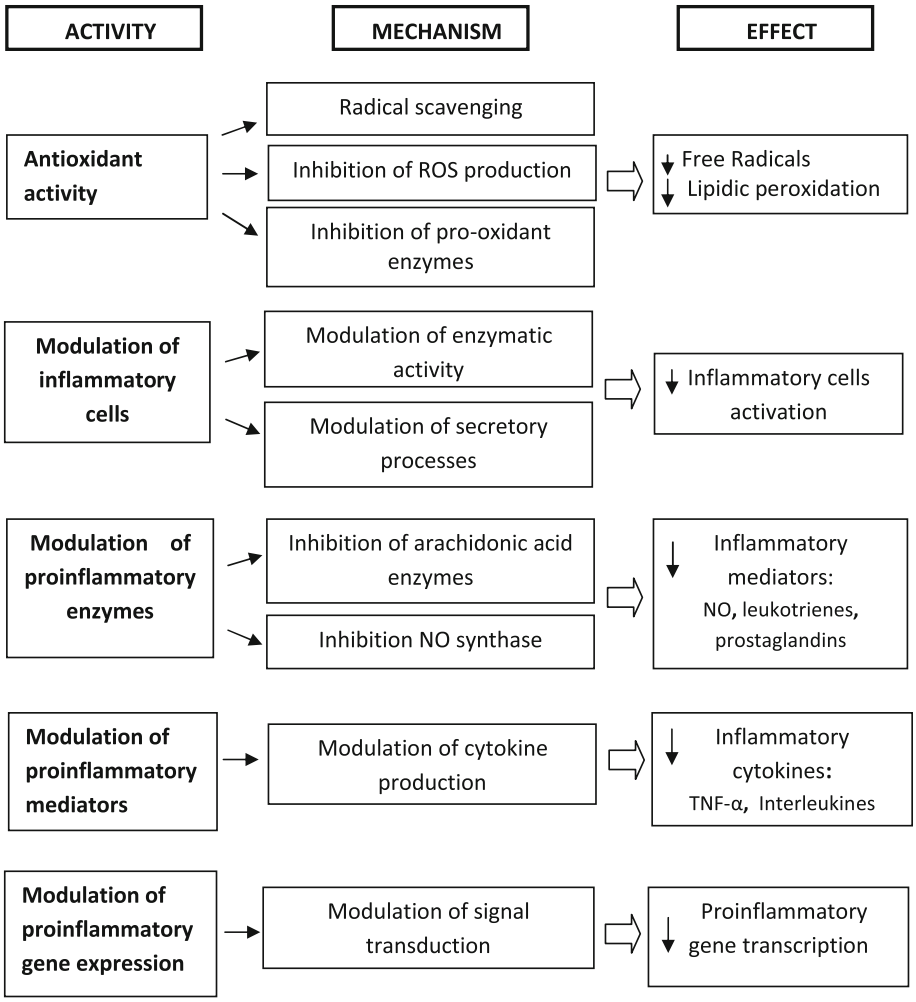

Fig. 1 Anti-inflammatory mechanisms of flavonoids

mechanisms have been identified explaining the way by which inflammatory states can promote cancer development (Figs. 1, 2).

Epidemiological studies have shown an inverse association between vegetables and fruits consumption and the risk of human cancers at many sites [128, 129]. Plant foods contain a wide variety of anticancer phytochemicals with potential bioactivities that may reduce cancer susceptibility. Among then, flavonoids are especially promising candidates for cancer prevention [130, 131]. Several studies in vitro and in animal models have demonstrated the effect of flavonoids in suppressing carcinogenesis [132–139].

Several mechanisms of action have been identified for flavonoids chemoprevention, including estrogenic/anti-estrogenic activity, antiproliferation, induction of cell-cycle arrest or apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of the host immune system, anti-inflammatory activity and changes in cellular signaling [140].

The cellular signaling pathways that regulate proliferation, survival and transformation of cells are of particular interest in current cancer research. Many of the molecular alterations associated with carcinogenesis occur in cell signaling pathways that regulate cell proliferation and differentiation. These pathways include several kinases such as MAPK, and protein kinases (PK), both of them, closely implicated in inflammatory processes. Abnormal activation or silencing of these kinases or their downstream transcription factors can result in uncontrolled cell growth, leading to malignant transformation [141]. Some flavonoids can modulate these pathways, which in turn regulates gene expression and favors the inhibition of carcinogenesis [97]. Table 2 summarizes some studies demonstrating anti-inflammatory mechanisms implicated in specific flavonoid chemoprevention [142–157].

Cancer is a largely preventable disease, namely, through an appropriate diet. Actually, since conventional therapeutic and surgical approaches have not been able to control the incidence of most cancer types, there is an

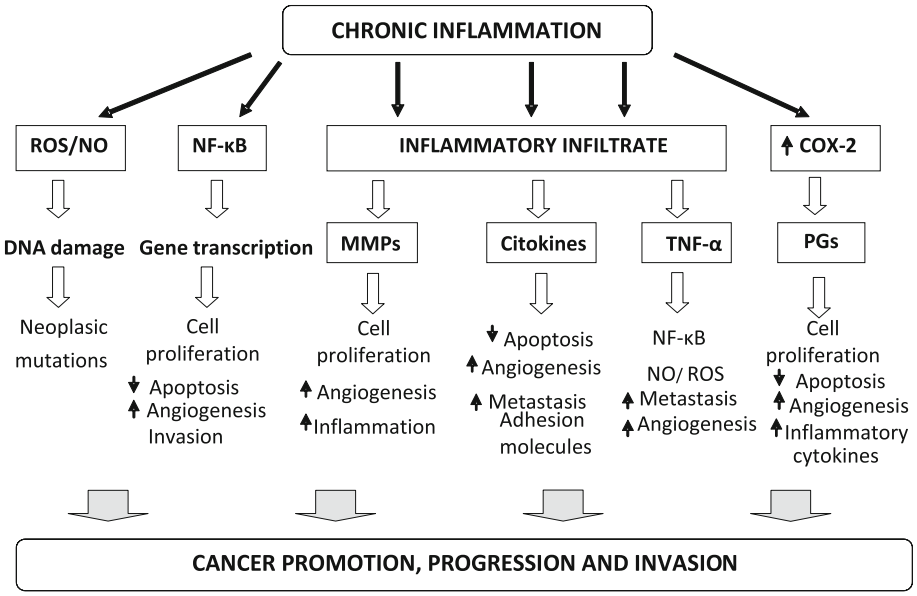

Fig. 2 Mechanisms of cancer promotion and progression by chronic inflammation

Table 2 Summary of studies demonstrating some of the anti-inflammatory mechanisms implicated in specific flavonoid chemoprevention

Mechanism	Compound	Cancer model	Reference
Antioxidant activity	Quercetin	Lung carcinogenesis	[142]
	Genistein	Neutrophils	[143]
COX-2 inhibition	Naringin	Colon carcinogenesis	[144]
	Tricin	Adenoma in APC^{min} mice	[145]
	Genistein	Human breast cancer cells	[146]
	Apigenin	UVB induced mouse skin tumors	[147]
Inhibition of PKC	Apigenin	Mouse skin tumors	[148]
	Luteolin	Skin tumor cell line	[149]
	Quercetin	Skin tumor cell line	[149]
Modulation of MAPK	Genistein	Prostate cancer	[150]
	Apigenin	Prostate cancer cells	[151]
	Apigenin	Breast carcinoma cells	[152]
Modulation of NF-κB	Morin	Different tumor cell lines	[153]
	Genistein	Prostate, breast and pancreatic Cancer cells	[154–156]
	Apigenin	Prostate cancer	[157]

urgent need to develop strategies in order to achieve this goal. In this way, dietary polyphenolic compounds such as flavonoids can be important candidates for chemopreventive agents [158]. However, more data from in-human studies are needed in order to draw definitive conclusions.

Flavonoids, inflammation, and cardiovascular disease

Cardiovascular disease is currently the main cause of death and illness in many countries. Inflammatory processes are common features in several cardiovascular conditions, such as atherosclerosis, acute coronary syndrome, myocardial ischemia-reperfusion injury and arterial restenosis [16].

Atherosclerosis, a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries, constitutes the single most important contributor to the growing burden of cardiovascular disease [159]. Recent advances in basic science have established a major role for inflammation in mediating all disease stages from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis [160].

One of the earliest events in the arterial wall in the initiation of atherosclerosis is the adherence of mononuclear cells to endothelium, which is triggered by a number of adhesion molecules such as P-selectin, E-selectin [161], vascular cell adhesion molecule-1 (VCAM-1) and

intercellular adhesion molecule-1 (ICAM-1) [162]. These molecules are expressed by endothelial and/or vascular smooth muscle cells upon proatherogenic stimuli such as oxidized LDL or oxidative free radicals [163, 164]. After monocytes and T lymphocytes bind to the surface of the arterial wall, they migrate into the subendothelial space, where they differentiate and are transformed into macrophages and foam cells. Transendothelial migration of leukocytes during the inflammatory process is triggered by chemotactic proteins such as monocyte chemoattractant protein-1 (MCP-1) [165] as well as by proinflammatory cytokines secreted by macrophages and T cells, such as TNF-α, IL-1, IL-6, [166, 167] and growth factors such as platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF) and transforming growth factor-β (TGF-β) [168]. These molecules contribute to atherogenesis by maintaining the inflammation inside the lesion and promoting the proliferation and migration of residential smooth muscle cells and the building of a dense extracellular matrix around them. The macrophage-lipid, T lymphocytes, smooth muscle cells and extracellular matrix enter a cycle of cell migration, proliferation and overproduction of fibrous tissue, leading to intermediate lesions and restructuring of the atheroma. All three classes of activated cells release proinflammatory mediators that induce the expression of cellular adhesion molecules, and gradually, the atheromatous plaque is formed [169]. Figure 3 shows the role of inflammation in the initiation and progression of atherosclerosis.

Inflammation is also involved in plaque rupture, which usually occurs in areas of sustained inflammation, and macrophage accumulation. Activated T cells may stimulate matrix metalloproteinases production by macrophages in the lesion. These proteolytic enzymes degrade the collagen of the protective fibrous cap, rendering the plaque susceptible to rupture [170]. Several cytokines may also upregulate the secretion of TNF-α, IL-1 and MG-CSF, contributing to the instability of the plaque [171].

Moreover, clinical studies have demonstrated systemic markers of inflammation to be strong predictors of clinical events, and specific treatments of atherosclerosis and its risk factor have been associated with reductions in inflammatory markers [172]. This link between inflammation and atherosclerosis provides a new target for future pharmacological agents that may slow the progression of atherosclerosis by inhibiting inflammation [173]. In this context, dietary flavonoids, as natural anti-inflammatory factors, may produce beneficial cardiovascular effects in human population, as supported by epidemiological data. Several prospective studies have reported inverse associations between flavonoid intake and cardiovascular disease incidence or mortality [174–177], whereas other studies have not [178, 179]. Recently, a prospective study of postmenopausal women showed that dietary intakes of flavanones, anthocyanidines, and certain foods rich in flavonoids were associated with a reduced risk of death due to coronary heart and cardiovascular diseases [180]. In a recent work, Hooper et al. performed a systematic review of the effectiveness of different flavonoid subclasses and

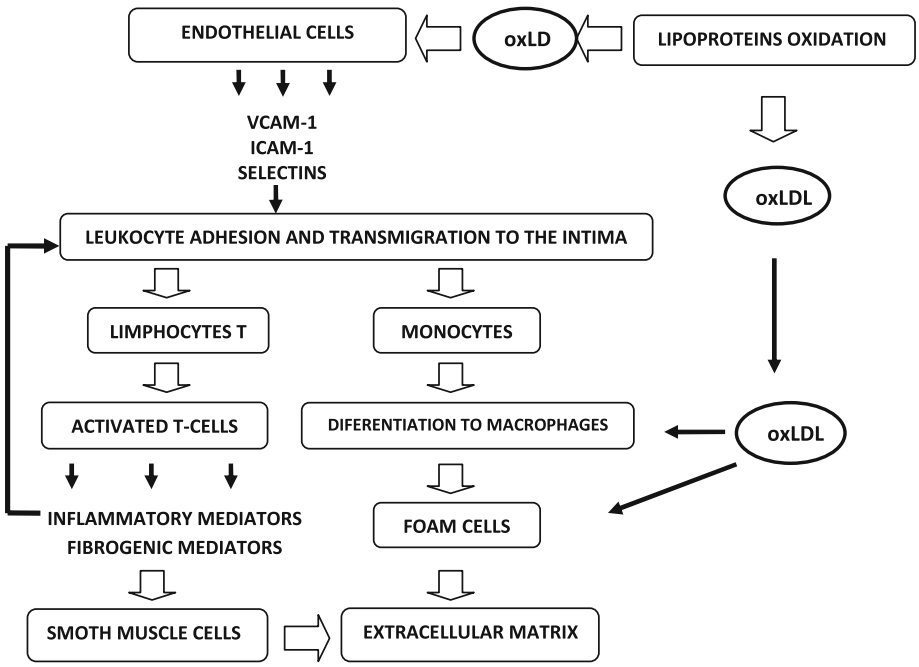

Fig. 3 Inflammation in the initiation and progression of atherosclerosis

flavonoid-rich food sources on CVD and risk factors. They concluded that although some flavonoid-rich foods may have some clinically relevant effects on CVD risk factors, there are limited data from intervention trials for other flavonoid subclasses consumed as part of a normal diet [181]. In addition to apparent benefits of flavonoid intake in the primary prevention, one study suggested that flavonoid intake in the form of tea might have benefit among individuals with established cardiovascular disease [182].

There are several mechanisms by which flavonoids may be protective against cardiovascular diseases, including antioxidant, anti-platelet, anti-inflammatory effects as well as increasing HDL, and improving endothelial function. Central to the pathogenesis of atherosclerosis is the oxidation of low-density lipoprotein (LDL), flavonoids have antioxidant effects and, additionally, some studies have shown that flavonoids decrease lipid peroxidation of biological membranes [183]. On the other hand, some mechanisms implicated in the anti-inflammatory effects of flavonoids may contribute to its cardiovascular protection, such as regulation of inflammatory mediators production. In an animal model, Droke et al. [184] demonstrated that soy isoflavone administration reduces the risk of cardiovascular disease associated with chronic inflammation, by down-regulating inflammatory mediators such as TNF-α at endothelial level. Furthermore, in vitro studies have revealed that dietary flavonoids such as apigenin, chrysin, kaempferol or quercetin, attenuate the expression of adhesion molecules in human aortic endothelial cells [185]. Isoflavones also may protect against inflammatory vascular disease by inhibiting monocyte–endothelial cell adhesion [186]. Flavonoids also may contribute to stabilization of the atheroma plaque, quercetin has been shown to be inversely associated with mortality from coronary heart disease by inhibiting the expression of metalloproteinase 1 (MMP1), and the disruption of atherosclerotic plaques [187].

All of these data suggest a great potential for dietary flavonoids as natural cardiovascular protectors. Continued studies of the biochemical mechanisms underlying cardiovascular diseases as well as biological effects of flavonoids will unveil new strategies for the treatment of such pathological conditions.

Conclusion

Excessive inflammation is considered as a critical factor in many human diseases, including two of the most extended burdens in the world: cancer and cardiovascular diseases. Epidemiological studies have demonstrated an inverse relationship between dietary flavonoid intake and prevalence and risk of these diseases. So that, flavonoids research have received much attention over the past years and a variety of potential beneficial effects have been elucidated. Their potent anti-inflammatory activity suggests the use of these compounds as potential prophylactic and therapeutic agents. However, most of the research involved in in vitro studies and the scarcity of data in bioavailability and in vivo models make it difficult to draw definite conclusions about the usefulness of dietary flavonoids. More bioavailability and intervention studies are needed in order to establish their effectiveness in the treatment of chronic diseases such as cancer and cardiovascular diseases.

Renewed scientific efforts will provide new insight into the anti-inflammatory activity of flavonoids, and eventually lead to development of a new class of natural anti-inflammatory agent.

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