Review

Molecular mechanisms underlying the anti-cancerous action of flavonoids

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ABSTRACT: Flavonoids are phenol compounds present in the pigments of fruit, vegetables, green tea and red wine. They have anti-allergic, anti-inflammatory, anti-microbial, anti-cancerous, anti-coagulant and anti-cholesterol effects. A few molecular mechanisms through which flavonoids exert their anti-cancer action are presented. One of the molecular mechanisms on which their anti-cancer action is based is their anti-oxidant activity which is exerted through the direct removal of the free radicals, the interaction with cell membranes, or the inhibition of xanthine-oxidase activity, an important source of ROS (radical oxygen species). Another is that by which flavonoids interact with the pathways signaling cell growth and apoptosis. Flavonoids interact with the signaling pathways for PI3-kinase, Akt/Pkb, tyrosine-kinase, P1KC and MAP-kinase. The same flavonoids may alter growth signaling by inhibiting receptor phosphorylation or by arresting growth factors binding to receptors. The activation of apoptosis genes is a characteristic of quercetin (a flavonol) which, in high concentration, induces thymidylate synthase – mediated apoptosis. In association with other drugs, flavonoids might prove useful in the treatment of cancer.

KEYWORDS*: flavonoids, anti-oxidant activity, signaling pathways, cell death*

INTRODUCTION

Flavonoids are phenol compounds present in the pigments of fruit, vegetables, green tea and red wine. Flavonoids were discovered by the Nobel prize winner Albert Szent Gyorgyi who called them vitamin P. Although many beneficial effects (anti-oxidant, anti-allergic, anti-inflammatory, anti-microbial, anti-cancerous, anti-coagulant and anti-cholesterol) on human health have been suggested, the research of flavonoids has hardly progressed after the discovery of "the French paradox". Currently, research advances in the direction of understanding cellular and molecular mechanisms by which flavonoids exert their effects on the human body. Flavonoids have a series of important actions and can interact with drug transport, interfere with cycline-dependent cell cycle regulation, inhibit protein glycosylation or affect the function of platelets [1].

Flavonoids have two aromatic rings (ring A and ring B) linked by a bridge composed of three carbon atoms (fig. 1). Depending on their state of oxidation and functional groupings, flavonoids are classified in flavons, flavonons, flavonols, isoflavons, catechines, anthocyanidines and calchones. So far over 5000 flavonoids have been identified while others are being discovered as we speak.

The main flavonoids are quercetin (extracted from onion and grapes), genistein (from soya beens), apigenin (from parsley and celery), luteolin (broccoli), epicatechin, proanthocyanids, kaempferol (broccoli, grapefruit), catechins (green tea), resveratrol (grapes), curcumin (curcuma). The citric bioflavonoids include hesperidin, quercitrin, rutin and tangeritin [2].

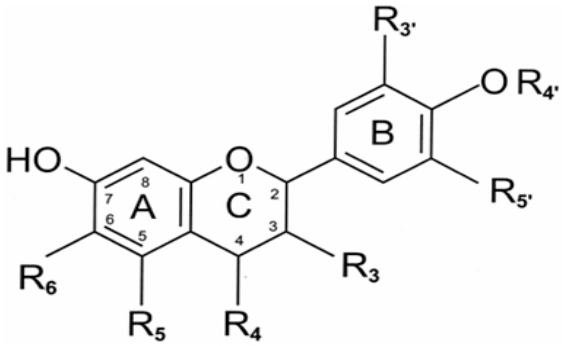

Fig. 1 The chemical general structure of a flavonoid.

The authors focus on the anti-cancerous effects of flavonoids and approach the mechanisms by which they exercise their action. Although the mechanisms that account for the anticancer action of flavonoids are not entirely understood, in vitro laboratory results point to some: the modulation of ROS production (reactive oxygen species), the arrest of cell cycle, the modulation of survival/proliferation pathways[3].

ANTI-OXIDANT EFFECT OF FLAVONOIDS

The most well defined property of flavonoids is their ability to act as anti-oxidants. Of all flavonoids, flavones and catechins have the strongest anti-oxidant effects. Like vitamin E, flavonoids contain structural chemical elements that may be responsible for their anti-oxidant activity. The position of hydroxyl groups and other factors in chemical structure of flavonoids contribute to their anti-oxidant activity and the removal of free radicals. The hydroxylation pattern of ring B may inhibit the activity of kinases and cell proliferation [4]. Moreover, a number of studies have rendered evident a structure – function relationship in which the anti-oxidant and anti-proliferation activity of flavonoids are dependent on clearly-defined structural models [5].

Mechanisms and sequences of events by which the free radicals interfere with cellular functions are still unclearly defined but one of the most important steps seems to be membrane lipid peroxidation leading to changes in the cell membrane and, consequently, to the variation of electric charges and the osmotic pressure.

To protect itself from ROS, the organism has developed several defense mechanisms which include enzymes, such as superoxide – dismutase, catalase, glutathione-peroxidase, and non- enzymatic duplicates, such as the ascorbic acid, glutathione, alpfa–tocopherol. In cell injuries the production of ROS increases, which leads to the depletion of endogenous molecules removing ROS and, consequently, to possible changes of the DNA. Therefore, an addition of exogenous anti–oxidant molecules is more that necessary in order to re-balance the ROS production and the anti–oxidant molecules. Flavonoids have an additive effect when associated with ROS-removing compounds. Their anti - oxidant ability is effected in different ways.

One way is the direct elimination of free radicals. Thus, flavonoids react directly with the free radicals, generating less reactive and more stable phenolic radicals. In other words, due to the high reactivity of hydroxyl groups existing in flavonoids, the free radicals become inactive. By removing the free radicals, flavonoids inhibit LDL oxidation (low density lipids) *in vitro*.

The second is the flavonoid interaction with cell membranes. The interaction with cell membranes includes: a) the separation of several nonpolar compounds within the hydrophobic inner membrane layer and b) the formation of hydrogen bonds between the polar bonds (heads) of lipids and the hydrophilic flavonoids at membrane interface. Flavonoids preferentially localized in the hydrophobic region of phospholipid bilayer initiates the formation of microdomains like rafts, while the molecules localized at polar interface of the bilayer can fluidify the membranes and initiate micellar structures [6]. The induction of such membrane changes may affect the oxidation rate of the membrane lipid and/or protein. The separation of some flavonoids in the hydrofobic core of membranes may decrease the access of oxidants, protecting structure and function of membranes[7]. This phenomenon, shown in experimental models, may help to understand cell – cell interaction and signal transduction.

Moreover, a phenolic compound can be oxidized into a quinoid compound that is similar to vitamin K, participating in the redox reaction. By chemical and/or ezymatic oxidation, quercetin, the most representative flavonoid, is converted into ortho-quinone leading to quinone-methides by isomerisation, reactive intermediates which alkilate the DNA. Recent results show the formation of quercetin-DNA/ quercetin-gltathione adductors in the cells exposed *in vitro* to tyrosinase and/or peroxidase. The cells with high levels of tyrosinase and peroxidase contain twice as many covalent adducts as the cells with undetectable levels of these enzymes. DNA-quercetin adducts are of transient nature, independent of DNA repair by excision, suggesting chemical instability of adducts [8]. The transient nature of DNA-created adducts might play a crucial role in *in vitro* genotoxicity if they are proved to have *in vivo* impact [9]

The interference of flavonoids with NO-synthase activity is another way to remove ROS. NO interacts with the free radicals and generates peroxynitrites that can directly oxidize ROS. When flavonoids are used as anti-oxidants, the free radicals are removed and the reaction of free radicals with NO can no longer take place. Furthermore, NO itself is a radical ousted by flavonoids. Another action mechanism of flavonoids is their interaction with different enzyme systems. Flavonoids, especially quercetin, silibin and luteolin, inhibit xanthine-oxidase, an important source of oxygen free radicals [10]. Flavonoids diminish the release of peroxidase. This reduction inhibits the production of ROS by neutrophils through their interfering with the activation of α1-anti-trypsin. An interesting effect of flavonoids on

the enzyme systems is their inhibitory effect on the arachidonic acid metabolism. The arachidonic acid release is a starting point for a general inflammatory response [11]

It has been suggested that, through their anti-oxidant action, flavonoids have a preventive role against stomach and colon cancers. The gastro-intestinal tract is continually exposed to ROS endogenously provided or resulting from food. In the stomach, the sources of ROS include mixtures of ascorbate and Fe^{2+}, lipid peroxides, cytotoxic aldehydes, isoprostanes and hydrogen peroxide. Nitrites from saliva and food are converted by the gastric acid into HNO_2 from which compounds derive that remove nitroso and amino groups from the DNA. Flavonoids also have the ability to inhibit deamination of nitrogenous bases of DNA structure by species derived from HNO_2, to upregulate the metabolism of toxins or anti-oxidant enzymes in the gastro-intestinal tract, to chelate the transition of metal ions and to decrease their anti-oxidant potential. Epidemiological studies show that a moderate consumption of red wine or cranberry juice might reduce the risk for cancer [12]. Certain studies show that the polyphenols existing in wine reduce the absorption of malondialdehyde, which is involved in atherosclerosis, cancer, diabetes and other diseases. Since their absorption is incomplete, the phenolic compounds reach the colon where, along with the products resulting from the bacterial metabolism in the colon, can have beneficial effects on the human body. Faeces contain micromolar amounts of flavonoids and large amounts of monophenols.

ANTI-PROLIFERATION EFFECTS OF FLAVONOIDS

Although initially it was suggested that the biological effects might depend on their anti-oxidant activity, cell cultures studies suggest that their biological effects result from the flavonids' ability to interact with different molecules along the cell growth signalling pathways and apoptosis. The flavonoids metabolits also retain their ability to interact with the proteins from the signalling pathways. The involvement of flavonoids in cell signalling might be one of the factors responsible for their anti-cancerous, vascular and cardio-protective activities [13].

It is already known that the oxidative stress generated by the imbalance between the production of reactive oxygen species (ROS) and the anti-oxidant defence system activity is responsible for cancer, aging, inflammation, neuro-degenerative diseases, etc. Anti-oxidant systems are insufficient and ROS-induced changes appear to be involved in carcinogenesis. ROS can modify the DNA and the division of damaged, unrepaired or wrongly repaired cells leads to mutations. If these mutations occur in oncogenes or tumor-suppressor genes, then the control of the cell cycle is disturbed and the cells can evolve to cancerous transformation.

Data from literature suggest that flavonoids target kinases phosphoring proteins in specific sites. Flavonoids interact with the signalling pathways of PI3-kinase (phosphoinositide 3-kinase), Akt/PKB (protein-kinase B), tyrosine kinase P1KC (protein-1 kinase C) and MAP (mitogen-activated protein) kinase. Stimulatory and inhibitory actions of flavonoids on these pathways affect cell functions by altering the phosphorylation of the target molecules and by modulating gene expression [14]. Flavonoids may alter growth signaling by inhibiting receptor phosphorylation or by blocking growth-factor receptor binding. In addition, flavonoids inhibit protein kinases Fyn and Lck, two representative members of SRC family of non-receptor kinases, involved in signalling in T cells transport [15]

PI3K catalyzes the production of phosphatidylinositol-3,4,5-triphosphat (PIP3) and phosphatidylinositol-4,5-bifosfat (PIP2). P1P3 can activate PDK1 ((phosphoinositide-dependent protein kinase 1) that activates Akt/PKB. Through its effects, PDK1 is involved in regulating cell growth, proliferation and differentiation, as well as cell migration and apoptosis. One of the most important targets of PI3K and PDK1 is Akt/PKB that plays a critical role in controlling cell survival and apoptosis. Akt inhibits apoptosis by inhibiting proteins Bad, Bcl-2 and caspases.

There are numerous data showing that flavonoids inhibit PI3K by direct interaction with their ATP binding site. Low quercetin concentrations can activate MAPK (ERK2, JNK1 and p38) path leading to the expression of survival genes (c-Fos, c-Jun) and of the genes involved in the anti-oxidant defense activity (detoxification enzymes, glutathione S-transferase, glutathione-reductase).

Flavonoids exert anticancer activity by blocking the cycle in various cells. In recent years, special attention was paid to disordered cell-cycle modulation of tumor cells due to different natural or synthetic agents. Among the natural agents we mention the flavonoids targeting regulator proteins (cycline-dependent

kinases and their inhibitors, protein p53 and Rb, E2Fs, ATM/ATR and surviving transition-controlling points G1/S and G2/M) [16]

Flavopiridol (a flavonoid extracted from *Dysoxilum binectariferum)* specifically inhibits CDK1 (cycline-dependent kinase1) and CDK2 [17].

Prostate cancer treatment with quercetin led to decreased cell proliferation and viability rates. Moreover, quercetin caused the apoptosis of cancer cells by down-regulating Hsp90 protein expression (heat shock protein 90) which, in turn, inhibited cell growth and caused the death of cancer cells [18]. In squamata cells, quercetin induced irreversible inhibition of cell growth and DNA synthesis and arrested the cell cycle in phase S [19]. Quercetin inhibited the growth of nasopharyngeal cancer cells by cell cycle arresting in G1/S phase [20]. Quercetin and kaempferol inhibited A549 cell proliferation derived from a lung carcinoma by activating ERK (extracellular signal-regulated kinase) [21]. Quercetin also arrested cell cycle in HepG2 cells by stimulating p21 and p53 protein expression [22].

Quercetin (248 μM) down-regulates the expression of mutant p53-protein to almost undetectable levels in the cell lines derived from breast cancer. This negative control was much weaker in cells with normal p53 gene. Quercetin, at a concentration of 70 μM, inhibited cell division and also tyrosine-kinase, an enzyme located near the cell membrane and involved in growth factors signal transduction to nucleus [23].

There are scientific data reports according to which flavonoids, mainly quercetin, have oral anticancer effects. Depending on dose and administration time quercetin inhibits the cell growth and the DNA synthesis. Quercetin induces SCC-9 cell necrosis at 24 to 48 hours of treatment and apoptosis when correlated with caspase 3 activity after 72 hours. Flow cytometry studies demonstrated that quercetin induces cell cycle blocking in phase S [19]. Quercetin inhibed B16-BL6 cell proliferation only after the latter had been treated for 48-hour. Quercetin caused apoptosis of B16-BL6 cells and inhibed the expression of Bcl-2 anti-apoptotic protein [24].

Flavonoids inhibit tirosine kinase receptors that are stimulated by epidermal growth factors.

APOPTOTIC EFFECTS OF FLAVONOIDS

Flavonoids induce mechanisms that kill cancer cells and inhibit cell proliferation. By modulating signalling pathways, polyphenols activate cell-death signals and induce apoptosis in pre-cancerous and maligne cells, inhibiting cancer development or progression.

Depending on quercentin concentration, cell death was induced by a 24-hour treatment of a LNCaP cells taken from human prostate. The inhibition of the Akt survival signal was triggered in the treated cells. The treatment of LNCaP with 100 microM of quercetin resulted in a rapid decrease in Ser 136 phosphorylation in Bad that is a Akt target. It has been shown that, while under treatment, Bcl-XL dissociates from Bax and associates with Bad. Quercetin decreases Bcl-XL/Bax ratio and increases translocation and multimerisation of Bax in the mitochondrial membrane; the translocation is accompanied by cytochrome c release, the clivage of caspases 3, 5, 9 and PARP [poli(ADP-ribose)polymerase]. Interestingly, at similar concentrations, quercertin does not affect cell viability or apoptosis rate in normal prostate epithelial cells [24]. Prolonged cell exposure to quercetin resulted in apoptosis mediated by the inhibition of thymidylate synthasev[19]. In CNE2 and HK1 cells, quercetin induced apoptosis in the first 24 hours of treatment and necrosis when the treatment was continued [20].

The green tea rich in catechins induces apoptosis and blocks the cell cycle in tumor cells but not in the normal ones. Green tea polyphenols affect the growth factors-mediated pathway, MAPK (mitogen-activated protein kinase)-dependent pathway as well as the ubiquitine/ proteasome degradation pathway [2].

HeLa cells treated with non-toxic flavonoid concentrations significantly sensitize TRAIL-induced cell death. Apigenin and ginestein increase TRAIL-mediated cytotoxicity in HeLa cells, but no such effect is obtained at kaempfeol and querectin [26]. Luteoline sensitizes therapeutically-induced citotoxicity by suppressing PI3K/Akt (phosphattidylinositol 3'-kinase), NF-kappa B (nuclear factor kappa B) and XIAP (X-linked inhibitor of apoptosis protein) and stimulates apoptotic pathways, including those that contain p53 protein [27,28]. At high concentrations, most flavonoids inhibit AP-1 activity (activator protein 1) via MAPK (mitogen – activated protein kinase) pathway [29].

Due to an increased public interest in alternative medicine, the products containing high doses of flavonoids can successfully complement conventional cancer therapy.

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