Molecular mechanisms underlying the anti-cancerous action of flavonoids

D.I. HERTZOG, OANA-SORINA TICA

University of Medicine and Pharmacy of Craiova, Department of Pharmacology

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].

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, alpha-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 hydrophobic 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 enzymatic 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-glutathione 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-antitrypsin. 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

It has been suggested that, through their anti-
oxidant action, flavonoids have a preventive role
against stomach and colon cancers. The gastro-
testinal 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
eroxide. 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 flavonoids’
ability to interact with different molecules along
the cell growth signalling pathways and
apoptosis. The flavonoids metabolites 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 phosphorhping 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).
PIP3 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 kinase 1) 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 inhibited B16-BL6 cell proliferation only after the latter had been treated for 48-hour. Quercetin caused apoptosis of B16-BL6 cells and inhibited the expression of Bcl-2 anti-apoptotic protein [24].

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

**APOTOTIC 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 malignant cells, inhibiting cancer development or progression.

Depending on quercetin 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 µM of quercetin resulted in a rapid decrease in Ser 136 phosphorylation in Bad that is an 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 cleavage of caspases 3, 5, 9 and PARP [poly(ADP-ribose)polymerase]. Interestingly, at similar concentrations, quercetin 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 synthase [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 kaempferol and quercetin [26]. Luteoline sensitizes therapeutically-induced citotoxicity by suppressing PI3K/Akt (phosphatidylinositol 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.

References