Apothosis in cancer and atherosclerosis: polyphenol activities

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Summary. Polyphenols have been demonstrated to have clear antioxidant activities in vitro. However, in complex biological systems, they exhibit additional properties which are yet poorly understood. Apoptosis is a genetically controlled and evolutionarily conserved form of cell death of critical importance for the normal embryonic development and for the maintenance of tissue homeostasis in the adult organism. The malfunction of the death machinery may play a primary role in various pathologic processes, leading to proliferative or degenerative diseases. Polyphenols can directly interact with specific steps and/or proteins regulating the apoptotic process in different ways depending on their concentration, the cell system, the type or stage of the pathological process. A growing body of in vitro evidence has provided interesting insights in the comprehension of the cellular and molecular mechanisms responsible for the modulation of apoptosis. However additional and harder studies are needed to better elucidate the mechanisms of action and the real in vivo effectiveness of polyphenols in order to propose them as potential candidates for chemoprevention and treatment of cancer and cardiovascular diseases.

Key words: polyphenols, apoptosis, carcinogenesis, atherosclerosis.

INTRODUCTION

Cancer and coronary heart disease are the most important disorders that cause alarming mortality and morbidity in humans. Research efforts of the last 30 years have shown that dietary habits and lifestyle may reduce their risk. Many foods because of their components, may provide protection against a variety of pathologies characterized by oxidative stress, including cancer and CVDs [1]. In particular it is well known that fruits and vegetables, rich in antioxidant compounds, are involved in prevention and protection against degenerative chronic diseases which are characterized by ROS overproduction and dysregulated apoptosis. Apoptosis is a genetically controlled and evolutionarily conserved form of cell death of critical importance for normal embryonic development and for the maintenance of tissue homeostasis in the adult organism [2]. The malfunction of the death machinery may play a primary or secondary role in various diseases, with essentially too little or too much apoptosis leading to proliferative or degenerative diseases, respectively. The machinery responsible for killing and degradation of the cell via apoptosis become activated through various stimuli. In addition, cell signalling pathways, mitogenic and stress responsive pathways are involved in the regulation of apoptotic signalling [3]. The fine-tuning of the balance between the pro- and anti-apoptotic fac-

Indirizzo per la corrispondenza (Address for correspondence): Claudio Giovannini, Centro Nazionale per la Qualità degli Alimenti e per i Rischi Alimentari, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: clagiovn@iss.it.
tors within each of these pathways in a cell leads to programmed cell death or survival.

Polyphenols, commonly contained in vegetables and fruits, represent more than 8000 different compounds, classified in different classes based on their chemical structure [4]. The most abundantly occurring polyphenols in plants are flavonoids and phenolic acids that account for 60% and 30%, respectively, of dietary polyphenols [5].

Several polyphenols have been demonstrated to have clear antioxidant properties in vitro, since they can act as chain breakers, radical scavengers and metal chelators depending on their chemical structures which also influence their antioxidant power [6]. A hierarchy has been established for the different polyphenolic compounds within each class on the basis of their capability to protect lipids, proteins or DNA, against oxidative injury. Many of their biological actions have been attributed, thus, to those antioxidant properties. On the other hand, pro-oxidant effects of polyphenols have also been described to have opposite effects on cell physiology processes. As antioxidants they improve cell survival, as pro-oxidants they may indeed induce apoptosis and cell death, and block cell proliferation.

Accumulating evidence indicates that polyphenols exhibit several additional properties in complex biological systems, mainly responsible for their protective effects. By virtue of these properties, among which the modulation of apoptotic process, polyphenols have been receiving more and more attention as therapeutic agents against cancer and cardiovascular diseases [7, 8]. Aim of this review is to focus the role of dietary polyphenols in modulating apoptosis, providing new insights into the molecular mechanisms underlying their protective effects.

APOPTOSIS

Apoptosis is characterized by a set of morphological changes including chromatin condensation, nuclear fragmentation, membrane blebbing and cell shrinkage [2]. Apoptosis can occur in mammalian cells by the extrinsic or intrinsic pathways (Figure 1). The extrinsic or death receptor pathway is activated when a specific ligand binds its corresponding cell-surface death receptor. Death receptors, such as tumour necrosis factor (TNF) receptor, TNF-related apoptosis-inducing ligand (TRAIL) receptor and Fas receptor, belong to the TNF receptor superfamily. In particular, the well-characterized Fas receptor (also called APO-1 or CD95) is activated by binding Fas ligand that leads to its trimerization and to the recruitment of Fas-Associated protein with Death Domain (FADD). The consequent conformational changes result in the binding of procaspases-8 to a supramolecular complex called Death-Inducing Signalling Complex (DISC) [9]. Caspase-8 activation can be blocked by cellular FADD-Like Interleukin-1β-converting enzyme Inhibitory Protein (c-FLIP). Conversely, caspase-8 can activate Bcl-2 interacting domain (Bid) (a proapoptotic member of the Bcl-2 family described below) which, in turn, can directly affect the mitochondrial membrane potential, thus interacting with the intrinsic pathway (Figure 1).

The intrinsic or mitochondrial pathway is activated by different agents, such as oxidants, toxicants, drugs or ionizing radiations, which all induce reactive oxygen species (ROS) overproduction and the onset of oxidative stress. Activation of the intrinsic pathway is accompanied by the translocation of cytochrome c from the mitochondrial intermembrane space into the cytoplasm. Cytochrome c, as well as Apoptotic protease-activating factor 1 (Apaf-1), endonuclease G and Apoptosis-Inducing Factor (AIF), are released from mitochondria after membrane potential collapse and function as proapoptotic factors. Cytochrome c, Apaf-1, dATP and procaspase-9 form a supramolecular complex termed ‘apoptosome’, that activates caspase-9 through autocatalysis. Both the mitochondrial-activated caspase-9 and the death receptor-activated caspase-8 cleave procaspase-3 generating the active caspase-3 that, in turn, activates other executor caspases, cleaves cytoskeleton and activates specific DNase. In addition, the activity of caspases is regulated by Inhibitors of Apoptosis Protein (IAPs) family that inhibit the cleavage of procaspases and/or the activity of caspases (Figure 1).

Among the molecules that exert their regulatory effect in determining cell fate, Bcl-2 family protein, p53 transcription factor and p66Shc represent important checkpoints which control the main steps of apoptotic process.

Bcl-2 family of proteins. Members of the Bcl-2 family of proteins are critical regulators of the mitochondrial membrane potential. Bcl-2 proteins localize, or translocate, to the mitochondrial membrane and modulate apoptosis by permeabilization of the inner and/or outer membrane, which leads to the release of cytochrome c, or by stabilizing barrier function. Most of the Bcl-2 family proteins are capable to interact each other, forming homo- or hetero-dimers, and functioning reciprocally as agonists or antagonists [10]. Maintenance or perturbation of mitochondrial membrane potential depends on the ratio between pro-apoptotic (Bax, Bad, Bak, Bid, Bcl-Xs) and anti-apoptotic (Bcl-2, Bcl-XL, Bag-1, Bcl-W) members of Bcl-2 family [11, 12].

p53. p53, also known as tumour protein 53, is a transcription factor that regulates cell cycle and apoptosis and hence functions as a tumour suppressor. In presence of DNA damage, p53 protein arrests cell cycle allowing time for cells to repair damaged DNA. When the damage cannot be successfully repaired, p53 acts as apoptotic signal. The loss of p53, or its mutations, decrease caspase activation and therefore apoptosis occurrence. p53 down-regulates anti-apoptotic genes. In addition, it can directly promote the translocation of the Fas death receptor from cytoplasmatic store to the plasma membrane [13], as well as the translocation of Bax protein from the cytoplasm to the mitochondria [14], thus allow-
The activation of extrinsic or intrinsic pathway, respectively. In addition, in addition p53 can displace Bax or Bid from pre-existing complexes with Bcl-XL, by binding to Bcl-XL itself, and consequently triggering apoptosis [15].

*p66Shc*. p66Shc is an oxidative stress sensor protein recently identified as an important cytoplasmic signal transducer that regulates the apoptotic response to oxidative stress [16, 17]. p66Shc has been identified as a splice variant of p52Shc/p46Shc, but unlike these variants, which are involved mainly in the transmission of mitogenic signals, p66Shc functions in the intracellular pathway by converting oxidative signals into apoptosis. The mechanisms of action of p66Shc have not been elucidated yet. However, it is accepted that it acts as a sensor for ROS production and as the downstream target of activated p53, in p53-dependent apoptosis [18]. It has been shown that the tumour suppressor p53 induces p66Shc up-regulation by increasing its stability and the increase of the p66Shc mitochondrial fraction. Expression of p66Shc is required for the ability of p53 to increase ROS production, leading cytochrome c release and consequently apoptosis. Finally, p66Shc has been demonstrated to be ROS producer within mitochondria because it acts as an oxido-reductase enzyme able to oxidize cytochrome c and to induce H$_2$O$_2$ production, causing the opening of permeability transition pores (PTP) and the release of the cytochrome c [19].

ROS are the most likely agents able to induce DNA damage in atherosclerosis, leading to apoptosis of
the cells of arterial wall. Likewise, oxidative stress has been found in various cancer cells and DNA mutations, which result from oxidative damage, represent the first step involved in mutagenesis and carcinogenesis. Cancer cells constitutively generate large, but tolerable, amounts of reactive oxygen species (ROS) suggesting that a certain level of oxidative stress may be required to maintain a balance between proliferation and apoptosis [20]. Apparently, ROS function as signalling molecules in the mitogen-activated protein kinase (MAPKs) pathways [21] to activate redox-sensitive transcription factors and responsive genes which are involved in the survival and proliferation of cells.

The MAPK signalling cascades include extracellular signal-related protein kinases (ERKs), JNK s/stress-activated protein kinases (SAPKs), and p38 kinases. The ERK pathway has been associated with the regulation of cell proliferation since it transmits signals initiated by growth promoters, and may ultimately foster cell growth and survival [22]. In contrast, the activation of JNK and p38 kinases is controlled by stress signalling, as oxidative stress, and has been associated with the induction of apoptosis [23, 24]. The balance between ERK and JNK activation is a key factor for cell survival since both a decrease of ERK and an increase of JNK are required for inducing apoptosis.

The activated MAPKs translocate to the nucleus, where they phosphorylate a number of substrates, including the transcription factors AP-1 and NF-κB which are linked to carcinogenesis and tumour promotion [22]. The activation of AP-1 and NF-κB promotes, in fact, survival and cellular proliferation, while their down-regulation sensitizes cells to apoptosis.

CARCINOGENESIS

In a stable mature tissue the rates of replication and cell death are balanced. Cell proliferation is regulated by checkpoints at the major stages of the cell cycle. If anyone of these checkpoints is overruled cell can be prone to natural or induced mutations and unable to repair the damaged DNA. Mutated cells which escape the apoptotic control can become the progeny of neoplastic cell population.

Carcinogenesis, a multistage process characterized by an accumulation of genetic alterations, could be divided in three main stages [25]. In the initiation stage, cells opposite to carcinogens by the activation of different enzymes. Phase I enzymes (e.g. cytochrome P450) react with carcinogen or xenobiotic to form a potent electrophile, mutagenic compound, which is responsible for DNA damage and mutations leading to the onset of cancer development.

Although phase II enzymes (e.g. glutathione transferase) can detoxify these compounds by forming water-soluble glutathione or sulfate conjugates which are easily eliminated by the body, this defence mechanism is often inadequate. The stage of tumour promotion is characterized by cell proliferation which is induced by the activation and/or over-expression of enzymes involved in the synthesis of nucleotides and DNA (ornithine decarboxylase), and in the regulation of the differentiation process (DNA polymerase or topoisomerase II). Moreover, during the promotion stage, ROS overproduction occurs, mainly due to the over-expression of pro-oxidant enzymes (e.g. cyclo-oxygenase, lypoxygenase), which leads to cell damages and further DNA mutations. In the progression stage, the final stage of carcinogenesis, the mutated cells proliferate in uncontrolled manner, and acquire a metastatic potential.

MODULATION OF APOPTOSIS BY POLYPHENOLS IN CANCER CELLS

Polyphenols can affect the overall process of carcinogenesis by several mechanisms. First of all, exogenous polyphenols supplied with the diet [26, 27], contribute to counteract oxidative stress occurrence and, in so doing, could contribute to the prevention of cancer onset and development. In fact they can modulate oxidative stress in cancer cells, thereby affecting signal transduction, activation of redox-sensitive transcription factors and expression of specific genes that influence cell proliferation and apoptosis.

In addition, a growing body of evidence indicates that polyphenols can directly interact with specific steps and/or proteins responsible for the regulation of apoptotic process such as the release of cytochrome c with subsequent activation of caspases-9 and caspases-3 [28-31], the increase of caspases-8 and t-Bid levels [30], the down-regulation of Bcl-2 and Bcl-XL expression, the enhanced expression of Bax and Bak [30, 32, 33] and the modulation of nuclear factor NF-κB [34].

Extensive data provide evidence for anticarcinogenic effects of resveratrol [35-37], a phenolic compound belonging to the class of stilbenes, found in many plant species and present in high amounts in grapes [38]. It causes cell cycle arrest and induces apoptosis in many human cancer cells, as prostate cancer cells [39], colon adenocarcinoma cells [40], esophageal carcinoma cells [41], breast cancer cells [42, 43], melanoma cells [44], pancreatic carcinoma cells [45], numerous human leukemia cells [46, 47], lung cancer cells [48]. The induction of apoptosis by resveratrol has been reported to be associated with increased caspase activity [40, 47-49], cell cycle dysregulation [50-52], decreased Bcl-2 and Bcl-XL levels, and increased Bax levels [49, 53]. Interestingly, these pro-apoptotic action has been reported to be frequently associated with the activation of p53 [43, 53]. A recently published paper demonstrated that the treatment of thyroid cancer cell lines with resveratrol caused an activation and nuclear translocation of ERKs that was associated with increased phosphorylation and accumulation of p53 protein and apoptosis induction [54].

Components of green and black tea, such as the flavonoid epigallocatechin-3-gallate (EGCG) and
other theaflavins, induced apoptosis and blocked cell cycle progression in a variety of cancer cells [32, 55]. The apoptosis induced by EGCG appears to be mediated by the activation of different signaling pathways probably depending on the cell type. At this regard, in human colorectal carcinoma cells, EGCG activates JNKs pathway [55]. On the contrary, in human prostate carcinoma cells, EGCG induced apoptosis, associated with stabilization of p53 and down-regulation of NFkB activity, resulting in a decreased expression of the anti-apoptotic protein Bcl-2 [56].

The hydroxybenzoic acid protocatechuic acid, one of the main metabolites of anthocyanins [57], also found in olives [58], brown rice [59] and tea [60], has been recently shown to induce apoptosis in human gastric adenocarcinoma cells through the Fas/Fas-L pathway, by activating JNK/p38 kinases. This event caused the mitochondrial translocation of Bax and the decrease of Bcl-2, triggering the cleavage of procaspases and resulting in the apoptosis of gastric cells [61].

Caffeic acid, a hydroxycinnamic acid found in many types of fruits and in high concentrations in coffee [5], induced apoptosis in human breast cancer cells [62] by activating pro-apoptotic factors such as Fas, Bax and caspases. Likewise it increased caspase-3 activity in stomach cancer, colon cancer and pro-myelocytic leukemia cell [63]. Furthermore, the treatment of glioma cells with caffeic acid induced the release of cytochrome c from mitochondria into cytosol and enhanced the expression of p53, Bax and Bak [64].

Pro-apoptotic activity, mediated by caspase-3 dependent mechanism, has been observed in oral squamous carcinoma cells exposed to different phenolic compounds derived from ginger, a common condiment for various foods and beverages [65, 66]. In human T-cell leukemia Jurkat cells, similar compounds activate mitochondrial pathway and alter the balance between the pro- and anti-apoptotic proteins, down regulating the anti-apoptotic Bcl-2 protein and enhancing the expression of the pro-apoptotic Bax [67].

Also curcumin, a polyphenolic compound derived from the rhizome of the plant Curcuma longa, induced apoptosis by suppressing the constitutive expression of Bcl-2 and Bcl-XL, and activating caspase-7 and caspase-9 in mantle lymphoma [68] and multiple myeloma [69] cell lines. Recently it has been demonstrated that curcumin induces apoptosis in prostate cancer cells, by down-regulating the expression of Bcl-2 and Bcl-XL and up-regulating the expression of p53, Bax, Bak, and Bim [70].

As described above, cancer cells require a certain level of oxidative stress, particularly those that are highly invasive or metastatic, and ROS can act as signalling molecules in the MAPK pathway [20].

It follows that if the excess of ROS can be scavenged by phenolic compounds which exert antioxidant activity, the oxidative stress-responsive genes can be suppressed and, consequently, cancer cell proliferation inhibited. On the other hand, polyphenols can induce the formation of ROS to achieve an intolerable level of oxidative stress in cancer cells. When the critical threshold for cancer cells to cope with oxidative stress has been reached, key cellular components, such as DNA, are irreparably damaged. In addition, genes involved in initiating cell cycle arrest and/or apoptosis are activated. Therefore, polyphenols can either scavenge the constitutive ROS or paradoxically generate additional amounts of ROS to inhibit the proliferation of cancer cells.

Both the mechanisms of action seem to be strictly linked to the phenolic concentration and the experimental conditions. It has been observed in fact that low or high concentrations of the same phenolic compound are responsible for antioxidant and pro-oxidant activity, respectively [20, 71].

Several studies suggest that polyphenols, in particular EGCG or resveratrol, can scavenge the constitutively high amounts of H$_2$O$_2$ in different cancer cells such as human epidermal keratinocytes, U-937 cells, Jurkat cells, HeLa cells, and H4 glioma cells [72-74]. Consequently they were able to block MAPK signalling, the activation NF-$\kappa$B and AP-1, and, ultimately, the expression of responsive genes that stimulate cancer cell proliferation. Additionally, resveratrol prevented NF-$\kappa$B activation induced by phorbol myristate acetate, lipopolysaccharide, okadaic acid, ceramide, and, most importantly, H$_2$O$_2$. Resveratrol had similar effects on the events which lead to the activation of transcription factors, as with the case of AP-1 in HeLa cells exposed to either PMA or ultraviolet radiation [73].

The flavone apigenin, abundantly present in fruits and vegetables, induced growth inhibition of human anaplastic thyroid carcinoma cells, probably by directly inhibiting the phosphorylation of MAPK, or alternatively, by scavenging H$_2$O$_2$, that activates the protein kinases [75].

As described above, under certain experimental conditions, polyphenols can paradoxically have pro-oxidant effects and generate ROS acting thus as cytotoxic and pro-apoptotic agent. This is the case for EGCG, quercetin, and gallic acid which generate H$_2$O$_2$, in a time- and concentration-dependent manner, when added to cell culture media resulting in stressful or cytotoxic effects [76]. Likewise, in Ha-ras gene-transformed human bronchial epithelial cells, a 24hr-treatment with 25 $\mu$M EGCG, or related tea catechins, induced apoptosis [77]. The death of the cells was attributed to H$_2$O$_2$, because the catechins induced formation of H$_2$O$_2$, and the addition of catalase prevented the apoptosis. In addition, the tea catechins decreased c-jun protein phosphorylation, which would be expected to lower AP-1 activity needed to transcriptionally activate some genes which promote cancer cell viability. Finally, the apoptosis induced by EGCG in human oral squamous carcinoma cells was attributed to the generation of H$_2$O$_2$ in cell culture medium [78].
The induction of intolerable amounts of ROS in cancer cells can initiate apoptosis through MAPK activation as demonstrated in U937 promonocytyc cells [79]. Similarly, tea catechins, including EGCG, induced ROS overproduction and activated MAPK before initiating caspase-mediated apoptosis [80]. It is worth of note that cancer cells, compared to normal cells, are more susceptible to be killed by anticancer drugs and polyphenols as well. This is probably because cancer cells are already close to a threshold for tolerating ROS. In fact, by using the same concentrations, phenolic compounds induced apoptosis in cultured cancer cells, but not in their normal counterparts as demonstrated for EGCG [81-84].

In agreement with these findings, internucleosomal DNA fragmentation was detected in A431 (human epidermoid carcinoma cells), HaCaT (human carcinoma keratinocytes), DU145 (human prostate carcinoma cell line) and L5178Y cell lines (mouse lymphoma cells), but not in NHEK cells (normal human epidermical keratinocytes) [85] after treatment with EGCG. Additionally, EGCG seems to possess a dual mechanism of action depending on the concentration, although it appears that this flavanol exerts its action in a selective manner in normal and cancer cells. In normal keratinocytes, EGCG enhanced proliferation at 0.5 μmol/L and did not affect cell growth at 50 μmol/L, while it decreased cell proliferation at both concentrations in a dose-dependent fashion in squamous carcinoma cell [81]. The dose-dependent inhibitory effect of EGCG on cell proliferation were also demonstrated in neuroblastoma SH-SY5Y cells, where low flavanol concentration (1 μmol/L) induced an anti-apoptotic response, while higher concentration (50 μmol/L) caused a pro-apoptotic effect [86].

It should be also taken in account that polyphenols can elicit different cellular responses depending on cell age. This has been clearly demonstrated in normal human primary epidermal keratinocytes by Hsu et al. [87]. The flavanol EGCG appeared to induce differentiation of immature keratinocyte after 24 h of treatment at concentrations of 15-200 μmol/L, while cellular proliferation was stimulated in aged keratinocytes (15-25 days) when epidermal cells were incubated for 1 day with the catechin at high concentrations (100-200 μmol/L). The authors suggested the use of this polyphenol in the treatment of wounds or certain skin conditions characterized by altered cellular activities or metabolism.

ATHEROSCLEROSIS

One of the main risk factors for coronary heart damage as well as other CVs is atherosclerosis. Atherosclerosis is an inflammatory process, triggered by the presence of lipids in the vascular wall, and encompasses a complex interaction among inflammatory cells, vascular elements, and lipoproteins through the expression of several adhesion molecules and cytokines. The pathophysiology of atherosclerosis is complex, involving both apoptosis and proliferation at different phases of its progression. Subendothelial retention of lipoproteins is the key initiating event in atherosclerosis, provoking a cascade of events that lead to the pathogenic response. In particular oxidatively modified LDL (oxLDL) are present in atherosclerotic lesions [39, 88, 89] and have been suggested to play a significant role in atherogenesis [90]. An elevated level of plasma LDL concentration leads to an increased traffic of LDL particles inside the artery wall [91]. LDL particles trapped within arterial wall are prone to progressive oxidative damage. Minimally modified oxidized LDL are responsible for the release of chemioactive factors by endothelial cells which initiate monocyte recruitment and promote their differentiation in macrophages, determining the occurrence of inflammatory process [92, 93]. Fully oxidized LDL, because of modification of apoprotein apoB, are recognized by scavenger receptors and internalized in macrophages which, by accumulating lipids, change into foam cells [91]. Oxidative modification of lipids and inflammation can differentially regulate the apoptotic and proliferative responses of vascular cells during the progression of the atherosclerotic lesion. There is increasing evidence that human atherosclerosis is associated with damage to the DNA of the cells of the vessel wall. DNA damage produces a variety of responses, including cell senescence, DNA repair or apoptosis. Apoptosis is frequently observed in endothelial cells, macrophages and vascular smooth muscle cells (VSMCs) in atherosclerotic plaques [94-96], and can directly contribute to the pathogenesis of cardiovascular diseases. The apoptotic endothelial cells become pro-coagulant, promoting platelet and neutrophil aggregation and thereby amplify the inflammatory response [96]. In addition, apoptosis induces up-regulation of inflammatory genes with release of biologically active cytokines, such as IL-1β, and the release of oxidized phospholipids capable of inducing monocyte-endothelial interactions. In advanced atherosclerotic plaques, up to 50% of the apoptotic cells are macrophages and this may promote core expansion and plaque instability. Both the inducers and the consequences of macrophage apoptosis are likely to be different between early and late lesions. In fact, pro-apoptotic factor derived from activated endothelial cells, such as TNFα, Fas-L and NO, may be more important in early lesions, whereas oxLDL, oxysterol, hypoxia/ATP depletion and the intracellular accumulation of unesterified cholesterol may be relevant in the more mature lesions. Many diverse factors cause VSMCs apoptosis, such as macrophage direct killing, via TNFα, Fas-L or NO, oxLDL and ROS production. Loss of VSMCs by apoptosis observed in the atherosclerosis plaques [95, 97] weaken the fibrous cap and predispose to
rupture leading to collagen exposure platelet activation, thrombosis and potential occlusion.

PROTECTIVE EFFECT OF PHENOLIC COMPOUND AND MODULATION OF APOPTOSIS IN ATHEROSCLEROSIS

Epidemiological and in vivo studies in humans have shown an inverse association between the consumption of polyphenols, or polyphenol-rich food, and the risk of cardiovascular diseases, suggesting protective effects of phenolic compounds [98-101].

Several mechanisms by which polyphenols may reduce risk for cardiovascular diseases have been proposed [102]. They affect plasma lipids and lipoproteins reducing plasma cholesterol and triglycerides. They also exert a protective effect on platelet function and haemostasis inhibiting platelet aggregation. Furthermore they control blood pressure and vascular reactivity promoting nitric oxide-induced endothelial relaxation. In conclusion they may oppose to the growth of atherosclerotic plaque by reducing the expression of adhesion molecules, exerting anti-inflammatory action and counteracting the macrophage-mediated oxidation of LDL.

In particular, there is substantial evidence that polyphenols can exert their protective effects by blocking early events which lead to atherosclerosis such as LDL peroxidation and oxLDL-induced apoptosis. Several phenolic compounds, e.g. those contained in green tea, red wine, extra virgin olive oil and liquorice root, have been demonstrated, in vitro, to inhibit macrophage cell-mediated oxidation of LDL and increase endogenous antioxidant defences [5, 26, 103, 104].

These findings are consistent with several in vivo studies that have demonstrated the capability of ingested polyphenols to ameliorate the oxidative status of subjects with increased risk for CVD [26, 105]. Extra virgin olive oil (EVOO) phenolic compounds, in particular hydroxytyrosol, oloreuropein, tyrosol, protocatechuc acid, vanillic acid, are responsible for antioxidant and protective effects. In human, EVOO consumption has been shown to reduce LDL oxidizability [106-111], in postprandial state [112] rather than in fasting state [112, 113]. However, while many studies have pointed out the anti-atherogenic effects exerted by polyphenols in protecting the vascular wall from oxidation, inflammation, platelet aggregation and thrombus formation, few data are available for their anti-apoptotic activity which can play an important role in preventing the onset and progression of atherosclerosis. There is in vitro evidence that polyphenols exert further protective effects against apoptosis mediated by oxLDL and hydrogen peroxide in different cell systems such as bovine aortic endothelial cells (BAEC) and fibroblasts [114], by affecting several proteins and signalling factors [115]. Specifically, it has been demonstrated that polyphenols can affect apoptosis by modulating the level of expression of anti-apoptotic (Bcl-2, Bcl-xL) or pro-apoptotic (Bax, Bid, Bak) proteins [116, 117]. On the other hand, delphinidin, an anthocyanidin contained in grapes, is able to inhibit the release of cytochrome c from mitochondria in endothelial cells by increasing eNOS expression via MAPK inhibitor-sensitve pathway [118]. Similarly, resveratrol has been shown to protect endothelial cells (HUVECs) against oxLDL-induced apoptosis by inhibiting cytochrome c release and activation of caspase-3 [119]. Kaempferol, another phenolic compound of red wine, inhibits apoptosis induced in VSMCs by 7β-hydroxycholesterol, which is a component of oxLDL [120].

Finally, phenolic compounds contained in EVOO, have been shown to counteract the oxLDL-induced cytotoxicity and apoptosis in murine macrophage J774A.1 cells by strengthening the endogenous antioxidant cell defences. This effect seems to be related more to the capability in inducing gene expression for GSH-related antioxidant enzymes, such as glutathione peroxidase and glutathione reductase, than to the antioxidant power of the compounds [121, 122]. Actually, it has been very recently suggested that the anti-apoptotic effect observed in the macrophages could involve the modulation of p66Shc expression by the EVOO phenols [123].

CONCLUSION

Apoptosis represents a protective mechanism against neoplastic transformation and development of tumours by eliminating genetically damaged cells or cells that may be inappropriately induced to proliferate by mitogenic and proliferative stimuli. On the other hand, dysregulated apoptosis of the arterial wall cells is involved in the occurrence of the complex sequence of events responsible for atherogenesis.

Polyphenols can exert different actions in modulating cell apoptosis; in fact, they can act as pro-apoptotic or anti-apoptotic agents depending on their concentration, the cell system, the type or stage of the degenerative process. A growing body of evidence provides new insights in the comprehension of the cellular and molecular mechanisms responsible for the modulation of apoptosis, by influencing signal transduction pathways and transcription factors. However, additional studies are still needed to better elucidate the mechanisms of action and the real in vivo effectiveness of polyphenols in order to propose them as potential chemopreventive candidates for cancer treatment and cardiovascular diseases.

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