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REVIEW ARTICLE

Mitochondria as a target for novel chemotherapeutic agents based on phenolic acids

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ABSTRACT

Every day, new molecules with antineoplastic potential are discovered. Unfortunately, most of these molecules lack cell-type specificity, and are unable to kill tumor cells any more efficiently than normal cells. Also, a high number of those molecules are very toxic. In order to design more effective chemotherapeutic drugs, it is important to understand the interaction between novel molecules and biological systems. Certain cellular components are particularly relevant in the context of specific targeting and mechanisms of action. Mitochondria are not only the major source of cell energy but are also important in the control of processes that culminate in apoptotic cell death. Particular aspects of mitochondrial physiology (e.g. the negative transmembrane electric potential) facilitate selective targeting by anti-cancer molecules. Such mitochondria-specific drugs are referred to as 'mitocans'. Among potential mitocans, phenolic acids are attractive candidates. Plant-derived phenolic compounds are widely consumed in a normal diet, especially in fruits and vegetables. Besides their antioxidant properties, phenolic acids have been reported to display antiproliferative activity by promoting selective induction of tumor cell apoptosis. In some cases, the molecule acts by triggering the mitochondrial pathway for apoptosis. Here we review the potential role of several phenolic acids and derivatives as anti-cancer agents, highlighting the role of mitochondria as a primary subcellular target for this class of compounds. The present review intends to raise awareness for this promising direction of research.

Key words

Antineoplastic drugs; Antioxidants; Hydroxybenzoic acids; Mitochondria

RÉSUMÉ

Des nouvelles molécules anticancéreuses potentielles sont découvertes quasi journellement. Malheureusement la plupart de ces molécules n'exercent pas de spécificité cellulaire et ne tuent pas plus les cellules tumorales que les cellules normales. Un grand nombre de ces molécules sont par ailleurs toxiques. Dans le but de développer des molécules anticancéreuses plus efficaces il est dès lors important de comprendre l'interaction entre de nouvelles molécules et les systèmes biologiques. Certains composants cellulaires sont particulièrement appropriés comme cibles spécifiques pour leurs mécanismes d'action. Les mitochondries sont la source principale d'énergie cellulaire, mais sont aussi importantes dans le contrôle des processus qui aboutissent à la mort cellulaire par apoptose. Certaines caractéristiques physiologiques des mitochondries, comme leur potentiel de membrane, permettent une attaque spécifique des mitochondries par des molécules anticancéreuses appelées «mitocans». Les acides phénoliques sont candidats mitocans très intéressants. Les composés phénoliques naturels sont abondamment présents dans l'alimentation, en particulier dans les fruits et les légumes. Outre leurs propriétés

antioxydantes il a été dans certains cas démontré qu'ils possédaient des propriétés antiproliférative par promotion d'une apoptose sélective par mécanisme mitochondrial. Dans cet article nous revoyons le rôle potentiel de certains acides phénoliques et de leurs dérivés comme agents anticancéreux, en insistant sur le rôle des mitochondries comme cible subcellulaire pour cette classe de composés. Cette revue de la littérature souligne l'importance de cette nouvelle voie d'investigation.

Mots clés

Médicaments anticancéreuses; Antioxydants ; Acides hydro-xybenzoïques; Mitochondries

RESUMEN

Todos los días se descubren nuevas moléculas con actividad antineoplásica. Sin embargo, la mayoría de ellas carecen de especificad celular, lo que se traduce en una incapacidad para eliminar a las células tumorales en forma más eficiente que a las células normales. Un número importante de estas moléculas también son altamente tóxicas. En el diseño de fármacos quimioterapéuticos más efectivos es importante entender la interacción entre las nuevas moléculas y los sistemas biológicos. Algunos componentes celulares son especialmente relevantes en el contexto de su especificidad y mecanismo de acción. Las mitocondrias, además de ser la principal fuente de energía celular, también son importantes en el control de la apoptosis. Algunos aspectos fisiológicos de las mitocondrias, p.ej. el potencial eléctrico de membrana negativo, podrían ser un blanco selectivo molecular de algunos anticancerígenos. Estos fármacos antimitocon-driales específicos son citados en la literatura como 'mitocanos'. Los ácidos fenólicos son candidatos atractivos para actuar como mitocanos. Los compuestos fenólicos derivados de plantas son ampliamente consumidos en la dieta diaria, especialmente en las frutas y los vegetales. Además de su actividad antioxidante, los ácidos fenólicos han mostrado actividad antiproliferativa promoviendo la inducción selectiva de la apoptosis de las células tumorales; en algunos casos, activando la vía mitocondrial de la apoptosis. En este manuscrito hemos revisamos el papel potencial de varios ácidos fenólicos y sus derivados como agentes anticancerígenos, enfatizando el papel de las mitocondrias como el principal blanco subcelular de esta clase de compuestos. Esta revisión intenta dirigir la atención a esta prometedora línea de investigación.

Palabras clave

Medicamentos antineoplásicos; Antioxidantes; Ácidos hidroxibenzoicos; Mitocondrias

INTRODUCTION

Significant progress has been made in cancer research in recent decades. Profound changes are occurring in several new experimental approaches currently under pursuit [1], including genetic strategies, recombinant biology, irradiation, and anticancer chemotherapy. Despite improved pre-clinical data, some cancers are not easy to control while others display a high rate of remission. Chemotherapy has been one of the strategies with a higher success rate, particularly when based on natural compounds. The use of phytochemical-type molecules has emerged as a promising and pragmatic clinical approach: besides reducing cancer risk, these molecules constitute a wide family of natural compounds with a considerable range of important properties such as low toxicity, low cost, and effectiveness when orally administrated [2].

Among phytochemicals, phenolic compounds [such as phenolic acids, stilbenes, curcuminoids, vanilloids, chalcones, and flavonoids (Figure 1)], are one of the most numerous and ubiquitous groups of plant metabolites, and an integral part of the human diet. Phenolic acids and derivatives are a particular phenolic class known to display a wide variety of biological functions: besides their high antioxidant capacity, phenolic acids have shown remarkable modulating properties in the carcinogenic process. In this context, phenolic derivatives have been under intense scrutiny, in some cases having a higher biological activity than their parent compounds [2].

The determination of cellular target(s) for these natural compounds, as well as the characterization of the corresponding molecular mechanisms of action, can lead to the design of new and more effective agents based on the chemical structure of the parent (lead) compound. One possible experimental strategy is the development of molecules capable of triggering the mitochondrial pathway for apoptosis. Targeting particular aspects of mitochondrial physiology may provide an effective strategy to selectively trigger apoptosis in cancer cells. In fact, there are several differences between normal and transformed cells concerning the main routes for ATP production: although neoplastic cells are mostly dependent on glycolysis for ATP generation, mitochondria are usually functional, with other roles on cellular physiology besides ATP production [3]. Mitochondria also participate in the regulation of intracellular calcium and iron concentrations, as well as in several biosynthetic pathways. Also, production of reactive oxygen species (ROS), which might be harmful if excessively produced, and more importantly, the regulation of cell death pathways, are major roles of mitochondria in cancer cells. Finding a strategy to destabilize mitochondrial homeostasis can lead to compromised integrity and to the elimination of malignant cells [4].

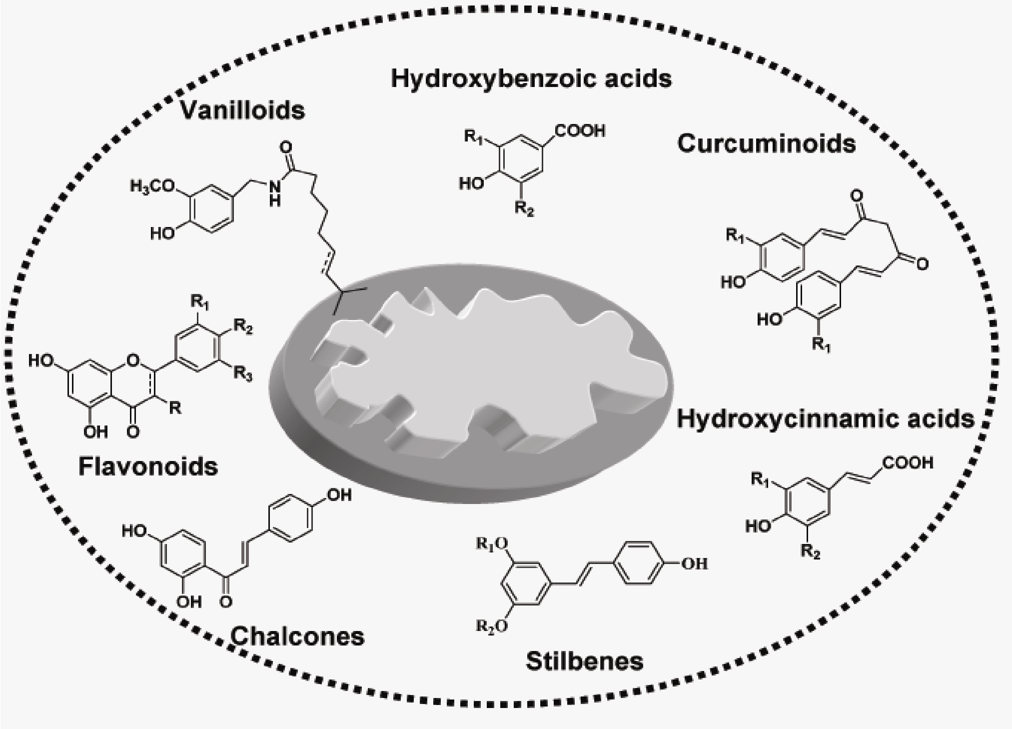

Figure 1 Examples of phenolic systems that can be used to investigate potential chemotherapeutic properties by inducing the mitochondrial pathway for apoptosis.

CHEMISTRY OF PHENOLIC ACIDS AND DERIVATIVES

Structural diversity is one of the main characteristics of phenolic systems, which constitute one of the most numerous and ubiquitous families of plant metabolites. Phenolic molecules display an impressive array of structures (more than 8,000 compounds have been described to date) classified into 10 different groups according to their chemical characteristics [5].

Apart from variations in the carbon skeleton, these compounds differ in the hydroxylation pattern of the phenolic ring, in the presence of alkyl ester or amide side chains, in the occurrence of stereoisomers, in the tendency for oligomerization in condensed phases, or in the conjugation to amines and sugars [6]. The biological activity of polyphenols is known to be largely by this structural wealth as much as by their antioxidant potency. In fact, their biochemical properties have been shown to be highly structure-dependent [7]: bioactivity varies considerably upon minor structural modifications, since these often induce significant conformational changes [8]. The bioavailability of phenolic compounds is directly related to their lipophilicity, which, in turn, is strongly influenced by chemical and structural preferences.

Several structure-activity relationship (SAR's) studies carried out on promising anticancer phenols [9] have shown that geometrical preferences are mainly determined by electrostatic factors, as well as by the formation of both intra- and intermolecular hydrogen bonds. Consequently, the most stable geometries display a clear preference for planarity. In the case of hydroxycinnamates, the presence of an ethylenic spacer in the carbon chain allows the formation of a conjugated system, strongly stabilized through π-electron delocalization.

An E-orientation of the aromatic ring and the C=O group relative to the carbon chain double bond was verified to be essential for the cytotoxic activity of caffeates and their derivatives [10]. The well known ability of phenolic compounds to form dimeric structures through OH•••O and/or CH•••O close contacts [11] should be taken into account, since their conformation and, consequently, their chemical properties vary considerably upon dimerization.

Furthermore, the biological activity of these systems has been reported to be affected by the introduction of alkyl groups in the carboxylic moiety of the molecule (ester or amide formation) [12]. Several reports describe a higher growth-inhibition and cytotoxic activity of phenolic esters relative to their acid counterparts [13]. Regarding the phenolic aromatic ring substitution pattern, trihydroxylated phenolic acids and esters were found to display a greater cytotoxicity, as well as antiproliferative properties, in comparison with their dihydroxylated analogues [14]. Preliminary studies on the antioxidant activity of trihydroxy- and dihydroxyphenolic acids showed that the former reveal a significantly higher activity [15]. Concerning the relevance of the degree of saturation of the carbon chain, it has been described that the presence of a double bond in cinnamates is associated with increased cell viability as opposed to the saturated compounds [16], which may be partially related to the increased antioxidant ability of the unsaturated phenols [17].

The biological behavior of phenolic systems may also be affected by their conjugation with other biomolecules (e.g. polyamines and glycosides), either by intracellular metabolic processes, or even by *in vivo* absorption and transport mechanisms. Since *in vivo* biological efficacy of phenolic compounds is influenced by bioavailability, knowledge of these types of structural factors is considered crucial for predicting the absorption versus concentration and metabolic profile.

On the other hand, a thorough understanding at the molecular level of the physicochemical properties (such as partition coefficients or redox behavior) and structural preferences including hydrogen-bonding motif of phenolic systems is essential for establishing the structure-property-activity relationships, allowing for optimization as antineoplastic agents with selective targeting.

Phenolic derivatives aimed for chemopreventive or chemotherapeutic applications can thus be rationally designed, through tailored chemical changes, in light of the structure-activity relationships or structure-property-activity relationships. Benzoic and cinnamic acids, as well as the phenolic flavonoids, have been extensively used as templates for this purpose, as the chemical modification of the lead phenolic compound is an important research area in medicinal chemistry.

APOPTOSIS

Transformed cells in cancer tissue are normally characterized by excessive proliferation, accompanied by the loss of control of cell processes. Activation of cell death by chemotherapy is beneficial if occurring in pre-neoplastic or tumor cells, but it may result in toxicity when taking place in normal cells. Of obvious clinical interest, some phenolic compounds were found to selectively induce time- and dose-dependent apoptosis only in immortalized (malignant) cells [18].

Apoptosis is a programmed cell death mechanism, finely regulated and genetically encoded. A typical phenotype consists in a progressive shrinkage of cells and formation of apoptotic bodies, as well as nuclear (chromatin condensation and nuclear fragmentation) and plasma membrane (e.g. phosphatidylserine exposure) alterations. Apoptosis can be monitored through the evaluation of several markers such as decreased mitochondrial membrane potential (mitochondrial depolarization), enhanced mitochondrial release of cytochrome c, increased caspase activation, chromatin condensation, or cleavage of Poli-ADP-ribose polymerase [19], among others. Apoptosis develops in different stages: 1) an initiation phase, which depends on the nature of the lethal signal; 2) a decision phase, characterized by equilibrium between pro- and anti-apoptotic molecules levels and 3) a common degradation phase, when the cell ceases its function [20]. However, cells that are deficient in cell death processes may not exactly follow this sequence. Programmed cell death can follow two major and well characterized routes, the extrinsic and the intrinsic pathway. The former is a death receptor-dependent pathway, consisting in the regulation of death receptors located on the cell surface. The intrinsic route is mitochondrial-dependent and includes the release of apoptotic signals from the mitochondrial inter-membrane space [21].

In the extrinsic pathway, a cell death signal is transduced within cells, either by the activation of the receptor-mediated process (absence of the corresponding ligands), or by the ligand-induced activation of receptors such as TNFR1, Fas, CD95/Fas (the receptor of CD95L/FasL) and of the tumor necrosis factor-α (TNF-α) receptor-1 [22]. In some cases, activated TNF receptors have been demonstrated to promote a different signalosome through NF-kB activation, leading to a release of inflammatory cytokines and to anti-apoptosis responses. TNF can also target angiogenesis, inducing tumor necrosis, overall having an antiproliferative activity [23]. If coupled to pro-apoptotic processes, death-inducible signaling complexes (DISC) are formed and cause activation of pro-caspase-8.It has been demonstrated that caspase-8 can activate pro-caspase-3, which then cleaves target proteins [21].

Also, in several cell types, caspase-8 first cleaves Bid (a Bcl-2 family protein) which, in turn, is directed to mitochondria or induces translocation, oligomerization and insertion of Bax and/or Bak, another Bcl-2 family member. Mitochondrial permeabilization follows, which leads to the release of several proteins from the mitochondrial intermembrane space, including cytochrome c. A cytosolic complex is formed in the presence of dATP, comprising cytochrome c, the apoptosis activating factor-1 (Apaf-1) and pro-caspase-9, resulting in caspase-9 activation, which triggers caspase-3 activity and signal amplification [22].

The intrinsic pathway is also characterized by the initial role of subcellular compartments such as the nucleus, lysosomes, the endoplasmic reticulum, or the cytosol itself, originating death-promoting stimuli that will act (directly or indirectly) on mitochondria. Permeabilization of the mitochondrial outer membrane (MOM) can occur, leading, as described above, to the release of intermembrane space proteins, including caspase activators such as cytochrome c, Omi/HtrA2 (Omi stress-regulated endoprotease/High temperature requirement protein A 2) and Smac/Diablo (second mitochondria-derived activator), as well as caspase-independent death effectors such as the apoptosis-inducing factor (AIF) and endonuclease G. AIF, endonuclease G and HtrA2/Omi are then directly translocated to the nucleus, triggering chromatin condensation and the appearance of high-molecular weight chromatin fragments [19].

Also, as previously referred, cytochrome c promotes activation of caspase-9, while Omi/HtrA2 and Smac/Diablo indirectly favor the caspase cascade by antagonizing the activity of the inhibitor of apoptosis proteins (IAPs), an endogenous caspase inhibitor. Besides IAP activation, another mechanism capable of rescuing cells from death involves Bcl-2 and Bcl-xL, which act by preventing the permeabilization of MOM and the release of proteins from the inter-membrane space [24]. Interestingly, studies have demonstrated that Bcl-2 proteins are strongly expressed in human breast cancer cells; in particular, Bcl-xL expression confers resistance to chemotherapy-induced apoptosis [25].

It has also been described that the intrinsic pathway for apoptosis can be triggered by the induction of the mitochondrial permeability transition (MPT), which involves the formation of protein pores that span the inner and outer mitochondrial membranes, resulting in mitochondrial depolarization, oxidative stress, and in some cases in outer membrane rupture due to matrix expansion, allowing pro-apoptotic proteins to escape to the cytosol [26]. The degree of MPT induction among the mitochondrial network, and the consequent decrease of mitochondrial ATP production, determines if apoptosis can be redirected to necrosis [27].

MITOCHONDRIA AS CHEMOTHERAPEUTIC TARGET

The discovery that mitochondria play a critical role in the process of cell death promoted a radical change in cell death research. Several oxidative stress-related diseases, in particular cancer, involve the understanding of mitochondrial-mediated apoptosis [3]. Mitochondria are thus potential targets for anti-cancer therapy. Different processes are suggested to interfere with vital mitochondrial functions in cancer cells. The vast majority of conventional anti-cancer drugs indirectly exploit the activation of intrinsic pathways in order to exert their cytotoxic action by using multiple activation routes (e.g. p53 or death receptors) [3]. Conventional anti-cancer agents, as well as numerous dietary components and micronutrients, have considerable potential for hindering *in vivo* deleterious oxidative processes and for inhibiting carcinogenesis through induction of apoptosis in neoplastic cells, in some cases via mitochondria-mediated pathways [28].

As discussed in the previous section, MOM permeabilization is generally regarded as a crucial control point in the commitment of a cell to apoptosis. During normal physiology, the MOM is considered freely permeable to solutes and small metabolites (approximately 5 kDa) due to the presence of the abundant protein voltage-dependent anion channel (VDAC), which allows the diffusion of such molecules through the MOM [29]. MOM rupture due to excessive osmotic influx can be lethal, not only by releasing caspase-activating molecules and caspase-independent death effectors, but also by initiating metabolic failure of mitochondria. For example, phenols such as gallic acid down-regulate Bcl-xL protein and activate Bak, Bax and Bad proteins, causing the release of mitochondrial cytochrome c to the cytosol, through MOM permeabilization [30].

Furthermore, oncogene products modulate MOM permeability by regulating Bcl-2 family proteins, which modulate the bioenergetic metabolite flux and putative components of the MPT pore. In this regard, phenolic acids may act through similar mechanisms. Several authors have reported that CAPE (caffeic acid phenyl ester) is cytotoxic against tumors, but not against normal cells, the mechanism involving mitochondria through altered Bcl-2 protein expression [31]. A number of other experimental chemotherapeutic agents have the opposite effect, acting on mitochondrial lipids and proteins and inhibiting MPT pores opening through direct binding to certain protein components [32].

A general rule is that antioxidants act as MPT inhibitors, decreasing or hindering the oxidative stress responsible for oxidation of proteins of the pore complex, and is a prime cause for the consequent inner membrane permeabilization [33].

In contrast to the MOM, the inner membrane is impermeable to the majority of ions, including protons. Membrane selectively is important for maintaining the proton gradient that is required for oxidative phosphorylation [21]. It is at the inner mitochondrial membrane that ATP synthesis occurs, through electron transfer coupled to proton ejection. Some potential anticancer molecules can also inhibit the respiratory chain, promoting mitochondrial failure and the release of proteins from the intermembrane space, increasing the generation of reactive oxygen species (ROS) and decreasing ATP generation. Ultimately, cell death occurs through mitochondrial failure [3].

The selective accumulation of promising anticancer molecules inside the mitochondria of tumor cells, rather than normal cells, is a key point in the design of novel molecules tested in new pharmacological strategies [34]. Several reports indicate that mitochondria have a higher transmembrane electric potential in tumor cells than in normal, non-neoplastic cells [35]. This difference has been used to induce a selective accumulation of specific molecules in the mitochondria of tumor cells, explaining its selective toxicity. We have reported a similar mechanism for the alkaloid berberine, which was found to inhibit proliferation in a murine metastatic melanoma cell line through a mitochondrial mechanism, mostly due to enhanced mitochondrial accumulation [36].

BIOLOGICAL ACTIVITY OF PHENOLIC ACIDS AND DERIVATIVES

Although naturally occurring phenolic acids have been the subject of numerous studies in cells, information on the molecular basis of their mechanisms of action at the sub-cellular level (e.g. mitochondria) is still somewhat scarce [37]. Phenolic compounds are usually associated with a beneficial effect on carcinogenesis principally due to their antioxidant activity, which mainly occurs by a free radical scavenging process [38].

Other pathways have been suggested, including the modulation of specific enzymes and signal transduction processes leading to cell proliferation arrest, cell cycle regulation, inhibition of DNA synthesis, or inhibition of angiogenesis and metastasis [39].

Phenolic acids can be subdivided into two major groups, hydroxybenzoic acids and hydroxycinnamic acids. Hydroxybenzoic acid derivatives include p-hydroxybenzoic, protocatechuic, vannilic, syringic, and gallic acid. These derivatives can be found in plants in free or bound forms, either as structural components of lignins and hydrolyzable tannins, or as a sugar derivatives and organic acids in plant foods. Hydroxycinnamic acids include p-coumaric, caffeic, ferulic, and sinapic acids. Like hydroxybenzoic acids, these molecules are present in the bound form, linked by ester bonds to components of the plant cell-wall such as cellulose, lignin and proteins [40].

Gallic acid and its esters are presently used as antioxidant additives in both food and pharmaceutical industry – propyl gallate (E-310) and octyl gallate (E-311) – since they are known to protect against oxidative damage induced by reactive oxygen, nitrogen and sulphur species [41]. Gallic acid derivatives have been described to cause apoptosis in tumor cell lines, to inhibit lymphocyte proliferation and to inhibit protein tyrosinase kinase (PTK's) activity [42].

Among hydroxycinnamic acids, caffeic acid is one of the most relevant and potent antioxidants found in the human diet, causing a significantly decrease in *in vitro* oxidative damage to DNA [43]. It has been demonstrated that the antioxidative capacity of caffeic acid attenuates the proliferation of Vascular Smooth Muscle Cells (VSMC) induced by angiotensin II stimulation, through a mechanism that involves inhibiting generation of reactive oxygen species generation and partially blockade of the JAK/STAT and the Ras/Raf-1/ERK1/2 cascades [44]. As potential anti-carcinogenic agent, caffeic acid inhibits cell growth during S-phase, causing further apoptosis in MCF-7 and T47D human breast cancer cells by activating pro-apoptotic proteins such as Bax and caspases [43] [45]. Alternatively, caffeic acid can also promote apoptosis in a number of cancer cell lines by inhibiting the NF-kB pathway [45].

Interestingly, caffeic acid is mentioned in some studies as a chemosensitizer, having a synergistic effect with doxorubicin treatment on MCF-7 human breast cancer cells [43]. Caffeic acid phenyl ester (CAPE) is a well-known phenolic acid derivative with promising potential, possessing antioxidant, anti-inflammatory, anti-viral, immunostimulatory, anti-angiogenic, anti-invasive, and anti-metastatic activities [46]. CAPE, together with other phenolic acid phenethyl esters, also possesses anti-cancer activity against some human cancer cells [47]. In human ME180 cervical cancer cells, CAPE promotes apoptosis mediated by down-regulation of Mcl-1 gene expression and activation of caspase-8 [46].

Moreover, CAPE was also reported to decrease cell viability in human U937 myeloid leukemic cells, in a dose and time-dependent manner. The apoptotic action of CAPE is mitochondrial-mediated and is accompanied by a decrease in Bcl-2 expression, increase of Bax expression, release of cytochrome c, activation/cleavage of caspase-3, and PARP cleavage [31].

Ferulic acid (4-hydroxy-3-methoxycinnamic acid), is a cinnamic acid structurally related to caffeic acid, which is present in rice bran and other plants. Food processing, such as thermal processing, pasteurization, fermentation, and freezing, contributes to the release of ferulic acid [40], which is rapidly absorbed and excreted as glucuronides or sulphates in men. Radical scavenging activity experiments showed that ferulic acid has antioxidant activity and that its esterification leads to a modification of its efficacy [48]. Ferulic acid derivatives present in the diet possess interesting biological effects including chemoprevention of colon and tongue cancers. One particular example is 3-(4'-geranyloxy-3'-methoxyphenyl)-2-trans-propenoic acid and its derivatives [49].

Curcumin is a phenolic derivative present in plants and is formed by two ferulic acids linked by a methylene group, in a diketone structure. The compound is the active ingredient of turmeric (Curcuma longa) and displays anti-inflammatory, antioxidative and anticarcinogenic properties, with an immense potential in cancer chemotherapy because of its effect in cell growth regulatory mechanisms. Analysis of its structure revealed that the presence of the beta-diketone moiety and phenolic groups in the structure have a direct contribution to its antioxidant behavior [50]. Curcumin exhibits growth inhibitory activity against prostate, colon and breast cancer [51]. Some studies evidence a selective growth-inhibiting effect of curcumin on transformed cell lines, as compared to non-transformed cell lines [52]. It was also previously demonstrated that the anticarcinogenic action of curcumin in colon have the involvement of the phenolic groups and the conjugated bonds in the central seven carbon chain [53].

Curcumin also affects various cell cycle proteins and checkpoints, leading to the down-regulation of some cyclins and cyclin-dependent kinases, as well as to up-regulation of cdk inhibitors, and to inhibition of DNA synthesis [54]. Of special relevance is the fact that curcumin can also induce apoptosis by targeting mitochondria, affecting p53-related signaling and blocking NF-kB activation, in a similar fashion to caffeic acid [50]. Prostate cancer cells can be sensitized with curcumin to TRAIL treatment, inducing both the extrinsic and the intrinsic pathways for apoptosis, which suggests that curcumin is a beneficial adjunct against chemoresistance to standard therapeutic drugs [54].

Phenolic compounds in general have been reported to affect nitric oxide (NO) production and its activity on physiologic functions. The phenolic acid 3,4-dihydroxy-phenylacetic acid (PAA) decreases the activity of NO synthase and consequently the possible production of endogenous reactive nitrogen species, besides arresting cells in the S-phase and inducing apoptosis of T47D breast cancer cells. The induction of apoptosis in this cell line occurs via FKHRL1 (FOXO3a) kinase pathway, while in MCF-7, a different breast cancer cell line, apoptosis is activated by NO via p53-associated pathways [45].

A second molecule, either a classic anti-cancer molecule (doxorubicin, taxol, cis-platin, etc) or an alternative molecule (a second phenolic acid or another natural phenolic compound), will be able to trigger signaling pathways that will ultimately end up affecting the already destabilized mitochondrial network. The outcome will be the release of mitochondrial proteins including cytochrome c or the apoptosis-inducing factor (AIF). These proteins are involved in the intrinsic pathway of apoptosis and the cascade of events will cause tumor cell death. It is noteworthy to point out that one advantageous aspect of mitochondrial physiology is that the transmembrane electric gradient (negative inside) can be used to drive the accumulation of lipophilic cations. Also, it is possible that at least part of the mitochondrial transmembrane electric gradient is maintained during the process of drug-induced cell killing, thus allowing mitochondrial ATP to be used during apoptosis. A complete collapse of the cell energy processes (mitochondrial oxidative phosphorylation and glycolysis) can drive the process of cell death from apoptosis to necrosis

FUTURE PERSPECTIVES

The efficacy of many clinically used chemotherapeutic drugs is often compromised by acquired chemoresistance by cancer cells. In fact, many cancer cells undergo mutations or loss of proteins that are intimately involved in cell death (e.g. p53 and Bcl-2 proteins). Phenolic derivatives present in fruits and vegetables, have long been recognized to display antioxidant and antiproliferative activities, thus being promising chemopreventive/chemotherapeutic compounds.

Exploiting the effect of these bio-antioxidants on cellular processes will lead to new chemopreventive and chemotherapeutic phenolic-based strategies that will hopefully be non-toxic to healthy cells. In order to overcome chemoresistance, mitochondria are an attractive biological target due to their major role in bioenergetics and vital signaling in mammalian cell. One new approach suggested is the use of compounds that specifically interact with the MOM, contributing to the release of pro-apoptotic proteins contained in mitochondria. Secondly, since the electrochemical gradient is apparently higher in mitochondria from cancer cells, lipophilic cations can be preferentially accumulated in the mitochondrial matrix [20].

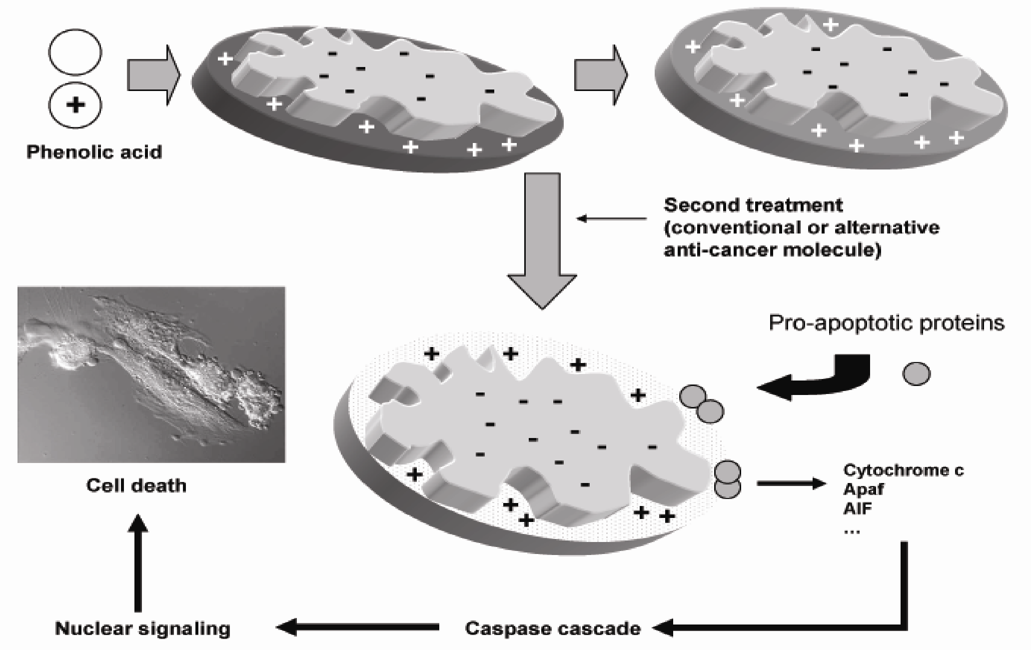

Figure 2 A proposed strategy in which the co-treatment of tumor cells with both a phenolic acid or derivative and a second molecule results in toxicity for the cancer cell. By altering the structure of the molecule (including modifying the polarization/charges), it is possible to direct the phenolic acid or derivative to mitochondria in order to create an initial destabilization in the mitochondrial membranes.

These strategies rely on the condition that derivatives from phenolic acids are small and positively charged, although neutral molecules can also, in theory, accumulate in mitochondrial membranes. A third method is based on the synergistic action of two or more compounds, the first of which is used to sensitize mitochondria to undergo failure and trigger apoptosis, if and when a second or a third molecule is added to the cell (Figure 2).

Although the presence of a positive moiety does not guarantee that the molecules will be accumulated by mitochondria, it is likely that once inside the cell lipophilic cations will tend to accumulate within organelles with a negatively-charged environment. Thus, the secret for success is to rationally design and synthesize phenolic acid derivatives that can be selectively accumulated by mitochondria in tumor cells, in such a way that the threshold for cytotoxicity exerted by a second molecule is significantly lowered (Figure 2). The mechanism of action results in a novel pharmacological strategy, coupling high efficacy against neoplastic cells to a low toxicity towards non-tumor cells. The advantage of using phenolic acids which are normally present in human diet is that the toxicity threshold is already understood

AUTHORS' PARTICIPATION

All authors participated in the composition of the text, figures and review of the manuscript.

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CONFLICT OF INTERESTS/DISCLAIMERS

PJO is member of the Editorial Board of the journal. No other conflicts of interest exist.

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