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EXPERT REVIEW

Resveratrol Mobilizes Endogenous Copper in Human Peripheral Lymphocytes Leading to Oxidative DNA Breakage: A Putative Mechanism for Chemoprevention of Cancer

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ABSTRACT Plant polyphenols are important components of human diet, and a number of them are considered to possess chemopreventive and therapeutic properties against cancer. They are recognized as naturally occurring anti-oxidants but also act as pro-oxidants catalyzing DNA degradation in the presence of metal ions such as copper. The plant polyphenol resveratrol confers resistance to plants against fungal agents and has been implicated as a cancer chemopreventive agent. Of particular interest is the observation that resveratrol has been found to induce apoptosis in cancer cell lines but not in normal cells. Over the last few years, we have shown that resveratrol is capable of causing DNA breakage in cells such as human lymphocytes. Such cellular DNA breakage is inhibited by copper specific chelators but not by iron and zinc chelating agents. Similar results are obtained by using permeabilized cells or with isolated nuclei, indicating that chromatin-bound copper is mobilized in this reaction. It is well established that tissue, cellular and serum copper levels are considerably elevated in various malignancies. Therefore, cancer cells may be more subject to electron transfer between copper ions and resveratrol to generate reactive oxygen species responsible for DNA cleavage. The results are in support of our hypothesis that anti-cancer mechanism of plant polyphenols involves mobilization of endogenous copper and the consequent pro-oxidant action. Such a mechanism better explains the anti-cancer effects of resveratrol, as it accounts for the preferential cytotoxicity towards cancer cells.

KEY WORDS cancer · comet assay · endogenous copper · pro-oxidant action · resveratrol

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INTRODUCTION

Epidemiological studies have suggested that human consumption of fruits and vegetables is associated with a reduced risk of cardiovascular disease and certain types of cancers (1). Resveratrol (3,4′, 5-trihydroxy stilbene) (Fig. 1) belongs to a class of compounds known as phytoalexins and has been isolated from several spermatophytes of which grapevine, peanuts and pines are the prime representatives. In recent years and earlier, a number of studies have appeared demonstrating that this molecule can affect several biological activities. Indeed, it has been proposed that resveratrol, which is present at high levels in red wine, exhibits a variety of biological actions, such as anti-infammatory (2), anti-platelet (3), and anti-mutagenic (4) effects, and has been shown to be an agonist for the estrogen receptor (5), a property which is relevant to its reported cardiovascular-protective properties. It has been found to confer resistance to plants against fungal infections (6) and inhibit DNA polymerase (7) and ribonucleotide reductase (8). Resveratrol has also been implicated as a cancer chemopreventive agent capable of inhibiting all three stages of chemical carcinogenesis, namely tumor initiation, promotion and progression (2). In agreement with the findings that cancer chemopreventive agents can induce apoptosis (9,10), resveratrol has been shown to induce apoptosis in human tumor cells (11,12). Most of the plant polyphenols, including resveratrol, possess both anti-

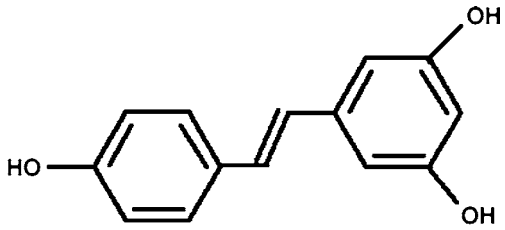

Fig. 1 Chemical structure of resveratrol.

oxidant as well as pro-oxidant properties (13,14), and we have earlier proposed that the pro-oxidant action of plant polyphenolics may be an important mechanism of their anti-cancer and apoptosis-inducing properties (15,16). Several other mechanisms have also been proposed to account for the anti-cancer properties of polyphenolic phytochemicals. Resveratrol is believed to be able to block the three steps in the process of carcinogenesis by inhibiting several molecular targets, such as kinases, cyclooxygenases, ribonucleotide reductases and DNA polymerases (17). Further, several plant polyphenols have also been shown to induce G1 phase arrest and to trigger mitochondrial-dependent, p53-dependent, ROS-dependent, bcl-2 sensitive apoptotic response in tumor cells (17,18). Resveratrol has been shown to induce activation of p53 accumulation and inhibition of NFkB. Other studies have emphasized the role of polyphenols as topoisomerase II poisons causing enhanced cellular DNA cleavage (19,20). A further evaluation of the literature would indicate that the subject of anti-cancer mechanisms of plant polyphenols is rather complex and controversial. However, it does appear that the anti-carcinogenic activities of plant polyphenols may be related but not entirely due to their anti-oxidative and above-mentioned properties. As already mentioned, a pro-oxidant action may be important in anti-cancer and apoptosis-inducing properties of these compounds. Further, it has been proposed that most clinically used anti-cancer drugs can activate late events of apoptosis (DNA degradation and morphological changes), and the essential signaling pathways differ between pharmacological cell death and physiological induction of cell death (21). Of particular interest is the observation that several plant polyphenols, including resveratrol, have been found to induce apoptosis in cancer cell lines but not in normal cells (11,22,23). Based on our own observations and those of others, we have proposed a mechanism for the anti-cancer properties of plant polyphenolics that involves mobilization of endogenous copper. As described below, such a mechanism would explain the preferential cytotoxicity of resveratrol towards cancer cells. Studies on chemopreventive and therapeutic plant-derived phytonutrients assume significance in view of the fact that such compounds exhibit negligible or low toxicity. Further, they may also act as lead compounds for the synthesis and development of novel anti-cancer drugs.

RESVERATROL IN CANCER CHEMOPREVENTION

Resveratrol was first described as a component in the root of *Polygonum cuspidatum*, a weed whose extract is well known in Asian medicine for its anti-inflammatory properties (24,25). Initially, the research on resveratrol gained momentum as a cardioprotective agent present in red wine, which was considered to explain the "French paradox." However, its potential as a cancer chemopreventive agent was realized after a paper was published by John Pezzuto and coworkers in the journal "*Science*" in 1997 (2). The study demonstrated that resveratrol was able to inhibit all three major stages (*i.e.* initiation, promotion and progression) of carcinogenesis. Since then, the molecule has attracted considerable attention for its anti-cancer properties. Resveratrol has been shown to cause growth inhibition and induce apoptosis in several cancer cells *in vitro*, including prostate, breast, skin, liver, pancreatic, lung and leukemic cancer cells (26–32). Further, the anti-cancer effects of resveratrol in *in vivo* tumor models have also been demonstrated (24,33–35). An important aspect of chemopreventive action of resveratrol that needs to be emphasized is its differential activity in selectively targeting cancer cells. Resveratrol has been shown to induce apoptotic cell death in tumor cells but not in normal cells (11).

ELEVATED COPPER LEVELS IN CANCER

The role of copper in both the etiology and growth of tumors has been extensively studied (36,37). Such studies were based on reports that copper distribution is altered in tumor-bearing mice, rats and humans (38–40). Several studies in the literature have shown that both serum and tumor copper levels are significantly elevated in cancer patients compared to healthy subjects (Table I). Moreover, there are a number of studies which have focused on determining the concentrations of four important elements: copper, zinc, iron and selenium in cancer patients. These studies showed that while zinc, iron and selenium concentrations were significantly lower in cancer patients, the copper concentrations were almost always found to be significantly elevated (2–3-fold) compared to age-matched samples from normal tissue (50,51).The reason for an increased copper concentration in tumors is not clearly understood. However, it is unlikely that this is the system's approach to rid itself of neoplastic cells, since the major copper-binding protein ceruloplasmin, which is also elevated in cancer cells (52), has been proposed to be an endogenous angiogenic stimulator (53).

Table I Serum and Tissue Copper Levels in Normal Individuals and Cancer Patients

Cancer	Normal copper level (μg/dL)	Cancer copper level (μg/dL)	Reference
Serum level			
Breast cancer	122.4	222.7	Yucel *et al.* (41)
Prostate cancer	84.1	124.0	Habib *et al.* (42)
Ovarian cancer	92.9	139.5	Chan *et al.* (43)
Leukemia	114.0	328.0	Carpenteiri *et al.* (44)
Colorectal cancer	98.8	165.0	Gupta *et al.* (45)
Lung cancer	143.0	188.2	Scanni *et al.* (46)
Tissue level			
Breast cancer	9.3	21.0	Rizk *et al.* (47)
Bladder cancer	1.5	2.8	Margalioth *et al.* (48)
Ovarian cancer	1.2	2.1	Margalioth *et al.* (48)
Leukemia	15.0	52.0	Carpenteiri *et al.* (44)
Colorectal cancer	1.7	2.7	Gupta *et al.* (45)
Stomach cancer	1.1	1.7	Yaman *et al.* (49)

A PRO-OXIDANT MECHANISM OF RESVERATROL IN CHEMOPREVENTION OF CANCER

Studies carried out in our laboratory (described in the succeeding sections) have proposed a novel mechanism for the cytotoxic action of resveratrol, which has implications for its chemopreventive properties against cancer.

Oxidative DNA Breakage by Resveratrol *In Vitro* in the Presence of Copper Ions

Most of the pharmacological properties of plant polyphenols are considered to reflect their ability to scavenge endogenously generated oxygen radicals or those free radicals formed by various xenobiotics, radiation, etc. However, some data in the literature suggests that the anti-oxidant properties of the polyphenolic compounds may not fully account for their chemopreventive effects (14,54). Studies in our laboratory have shown that several polyphenols, including resveratrol (13,55–59), cause oxidative strand breakage in DNA either alone or in the presence of transition metal ions such as copper. Studies by Liu and coworkers (60) demonstrated that resveratrol, as well as certain of its synthetic analogs, namely 3,4,4-trihydroxy-*trans*-stilbene, 3,4-dihydroxy-*trans*-stilbene, 3,4,5-trihydroxy-*trans*-stilbene, which are generally effective anti-oxidants, can switch to pro-oxidants in the presence of Cu(II) to induce DNA damage. Most plant polyphenols possess both anti-oxidant as well as pro-oxidant properties (12,13), and we have proposed that the pro-oxidant action of plant polyphenolics may be an important mechanism of their anti-cancer and apoptosis-inducing properties (14). Such a mechanism for the cytotoxic action of these compounds against cancer cells would involve mobilization of endogenous copper ions and the consequent pro-oxidant action. Using a cellular system of lymphocytes isolated from human peripheral blood and alkaline single-cell gel electrophoresis (comet assay), we have confirmed that the resveratrol–Cu (II) system is capable of causing DNA degradation in cells such as lymphocytes (59).

Further, the resveratrol-induced DNA degradation in lymphocytes is inhibited by scavengers of ROS and neocuproine, a Cu(I)-specific sequestering agent. Copper is an important metal ion present in chromatin and is closely associated with DNA bases, particularly guanine (61,62). It is also one of the most redox active of the various metal ions present in cells. Evidence deduced in our laboratory has shown that polyphenols do not only bind copper ions but also catalyze their redox cycling (57). A mechanism was proposed which involves the formation of a ternary complex of DNA–polyphenol–Cu(II) (56,63). A redox reaction of the compound and Cu(II) in the ternary complex may occur, leading to the reduction of Cu(II) to Cu(I), whose reoxidation generates a variety of ROS. Thus, the pro-oxidant action of polyphenols requires the presence of molecular oxygen (55). Resveratrol reduces Cu(II) to generate Cu(I) as evidenced by the formation of a Cu(I)-bathocuproine complex absorbing at 480 nm (Fig. 2), and the stoichiometry of Cu(I) production by resveratrol does not show a clear maximum absorption plateau, suggesting a possible redox recycling of copper ions in the reaction. These findings demonstrated that the resveratrol–Cu(II) system for DNA breakage is physiologically feasible and could be of biological significance.

Oxidative DNA Breakage by Resveratrol in Human Peripheral Lymphocytes and Lymphocyte Nuclei

Using the comet assay, our laboratory has shown that resveratrol causes DNA breakage in isolated human

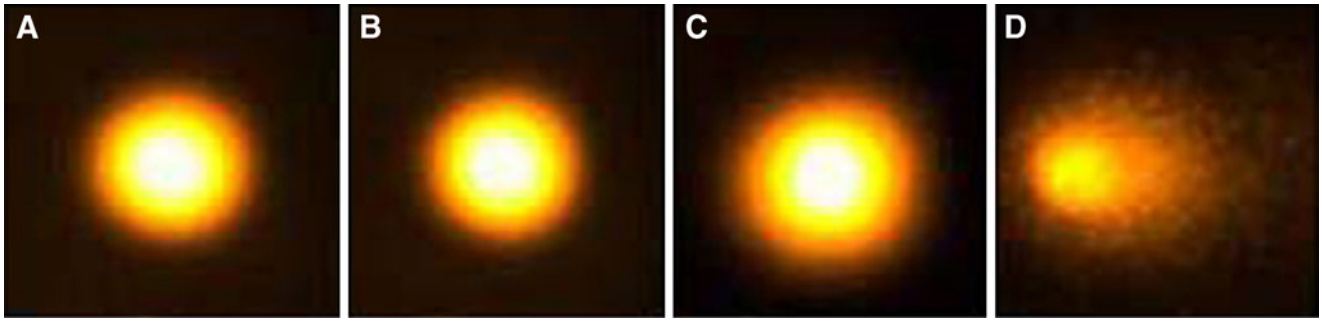

Fig. 2 Alkaline single-cell gel electrophoresis (comet assay) of human peripheral lymphocytes showing comets (100×) after treatment with different concentrations of resveratrol: **A** untreated, **B** 50 μM, **C** 100 μM and **D** 200 μM.

peripheral lymphocytes (64). Photographs of comets seen on treatment with different concentrations of resveratrol are shown in Fig. 3. At 50 and 100 μM concentrations, resveratrol did not damage the lymphocyte DNA to any significant extent, whereas at 200 μM concentration, a comet with a tail indicative of DNA breakage was observed. The results demonstrate that resveratrol alone is capable of DNA breakage in lymphocytes. Such cellular DNA breakage is inhibited by Cu(I) sequester neocuproine, indicating the involvement of intracellular copper ions in the DNA breakage reaction. As copper is an essential component of chromatin, we have also studied the resveratrol-induced cellular DNA breakage in a permeabilized cellular system (65), which allows direct interaction of resveratrol with nuclei by eliminating the cell membrane and cytoplasmic barriers. It may be noted that such permeabilized cells contain only cell organelles attached to the residual cytoskeleton. Thus, in a permeabilized system, considerably greater DNA degradation as compared with intact lymphocytes is expected to be observed. This is indeed found to be the case, as shown in Fig. 4. Table II gives the results of an experiment where the effect of three scavengers of ROS has been tested on resveratrol-induced DNA breakage in whole and permeabilized lymphocytes. SOD and catalase remove superoxide and H_2O_2, respectively, and thiourea is a scavenger of several ROS. All three cause significant inhibition of DNA breakage induced by resveratrol in both systems, as evidenced by decreased tail lengths (64). We conclude that superoxide anion and H_2O_2 are essential components in the pathway that leads to the formation of hydroxyl radical and other species which would act as proximal DNA cleaving agents. It is further suggested that a similar mechanism mediated by ROS is responsible for DNA breakage in whole cells as well as in cell nuclei.

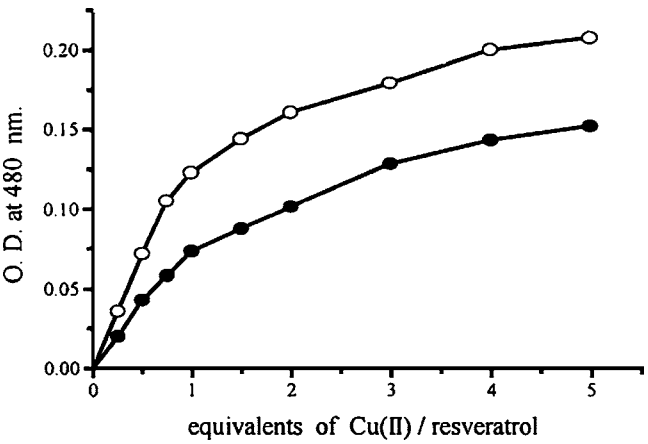

Fig. 3 Detection of stoichiometry of resveratrol and Cu (II) interaction. The difference in absorbance at 480 nm of samples with and without added Cu(II) is plotted *vs.* equivalents of Cu(II) per molar equivalent of resveratrol. Concentration of bathocuproine used was 300 mM in all cases. Concentrations of resveratrol used were (●) 5 mM and (○) 10 mM. All the points represent triplicate samples, and mean values have been plotted. $P < 0.05$ by comparison with samples in the absence of reveratrol.

Mobilization of Nuclear Copper by Resveratrol

In a previous study (64), we showed that the resveratrol-induced degradation of cellular DNA is inhibited by neocuproine, which is a Cu(I)-specific chelating agent and is membrane-permeable (66). In the experiment shown in

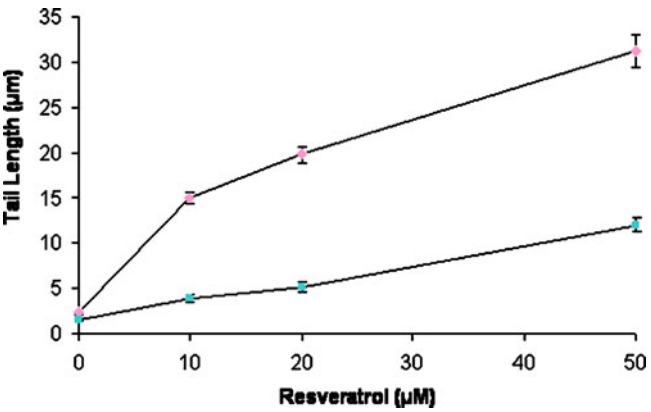

Fig. 4 A comparison of DNA breakage in intact lymphocytes (♦) and permeabilized lymphocytes (■) using increasing concentrations of resveratrol. Values reported are ± SEM of three independent experiments. $P < 0.01$ by comparison with control (in the absence of resveratrol).

Table II Effect of Scavengers of Active Oxygen Species on Resveratrol-Induced DNA Breakage in Whole Lymphocytes and Permeabilized Lymphocytes

Dose	Comet tail length (μm)	Inhibition (%)
Whole lymphocytes		
Untreated	1.22 ± 0.08	–
Resveratrol (150 μM)	20.84 ± 1.28^{b}	–
+SOD (100 μg/ml)	$7.05 \pm 0.25^{*}$	66
+catalase (100 μg/ml)	$8.86 \pm 0.29^{*}$	57
+thiourea (1 mM)	$11.93 \pm 1.01^{*}$	45
Permeabilized lymphocytes[a]		
Untreated	2.53 ± 0.03	–
Resveratrol (50 μM)	30.48 ± 2.33^{b}	–
+SOD (100 μg/ml)	$11.02 \pm 2.05^{*}$	64
+catalase (100 μg/ml)	$13.31 \pm 1.95^{*}$	56
+thiourea (1 mM)	$9.68 \pm 1.21^{*}$	68

All values represent S.E.M. of three independent experiments.

[a] Concentration of resveratrol used in the permeabilized system was considerably lower, as this was enough to generate significant tail length.

* $P < 0.05$ when compared to control[b].

Fig. 5, we have also used bathocuproine disulphonate (the water-soluble membrane-impermeable analog of neocuproine), iron chelator desferrioxamine mesylate and a zinc chelator histidine to show that, whereas neocuproine inhibits resveratrol-induced DNA breakage in intact lymphocytes, bathocuproine, desferroxamine mesylate and histidine are ineffective in causing such inhibition (Fig. 5a). It is well known that nuclear pore complex is permeable to small molecules. When the two copper-specific chelators (both of which would be able to permeate the nuclear pore complex) were tested for DNA breakage inhibition in permeabilized cells, both the copper sequestering agents were found to inhibit DNA breakage in a dose-dependent manner (Fig. 5b) (67). Further, chelators that specifically bind iron and zinc were again ineffective. From the results, we conclude that the cellular DNA breakage by resveratrol involves nuclear copper and that Cu (I) is an essential intermediate in this reaction.

It has been shown that polyphenols auto-oxidize in cell culture media to generate H_2O_2 that can enter cells/nuclei, causing damage to various macromolecules (68). In order to exclude this possibility in our system, we have determined the resveratrol-induced formation of H_2O_2 in the incubation medium of isolated nuclei (0.4M phosphate buffer) (65) and also compared it with a known generator of H_2O_2, namely tannic acid (69). The results showed that the rate of H_2O_2 formation by resveratrol was almost negligible, but that of tannic acid was quite significant. However, the DNA breakage efficiencies of the two polyphenols followed a reverse trend where resveratrol was found to be considerably more effective than tannic acid (67). It is therefore indicated that the nuclear DNA breakage observed in our studies is not the result of extraneous generation of ROS. We thus presume that the lymphocyte DNA breakage is the result of the generation of hydroxyl radicals and other ROS *in situ*.

Evidence for the Pro-oxidant Action of Polyphenols as an Important Mechanism for Their Anti-cancer Properties

We give below several lines of indirect evidence from our own work and that in literature, which strongly suggest that the pro-oxidant action of plant-derived polyphenolics rather than their anti-oxidant activity may be an important

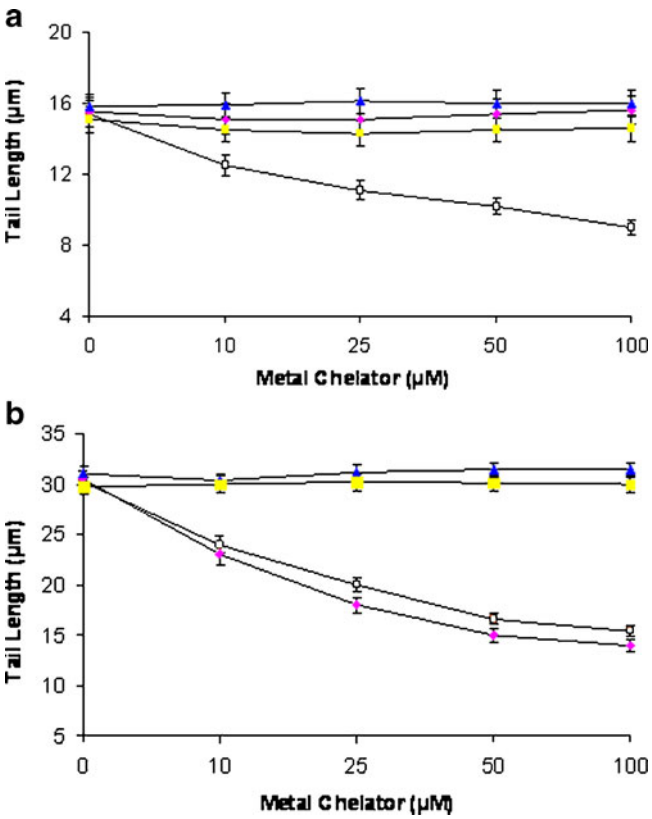

Fig. 5 **a** Resveratrol (150 μM) induced DNA degradation in lymphocytes in the presence of metal chelators: copper chelators, neocuproine (□) and bathocuproine (♦); iron chelator, desferrioxamine mesylate (■); and zinc chelator, histidine (▲). The concentration of chelators used was as indicated, and incubation was performed for 1 h at 37°C. Values reported are ± SEM of three independent experiments. $P < 0.01$ by comparison with control (in the absence of chelators). **b** Resveratrol (50 μM) induced DNA degradation in permeabilized lymphocytes in the presence of metal chelators: copper chelators, neocuproine (□) and bathocuproine (♦); iron chelator, desferrioxamine mesylate (■); and zinc chelator, histidine (▲). The concentration of chelators used was as indicated, and incubation was performed for 1 h at 37°C. Values reported are ± SEM of three independent experiments. $P < 0.01$ by comparison with control (in the absence of chelators).

mechanism for their anti-cancer and apoptosis-inducing properties:

(1) Apoptotic DNA fragmentation properties of several anti-cancer drugs (70,71) and γ-radiation (72) are considered to be mediated by ROS. It may also be mentioned that doxorubicin-induced apoptosis in human osteocarcinoma Saos-2 cells is mediated by ROS and is independent of p-53 (73). Interestingly, certain properties of polyphenolic compounds, such as binding and cleavage of DNA and the generation of ROS in the presence of transition metal ions (56), are similar to those of certain known anti-cancer drugs (74).

(2) Structure activity studies carried out in our laboratory with gallic acid (a structural constituent of tannic acid) indicate that if two of the three hydroxyl groups are methylated (syringic acid), the DNA degrading capacity (both *in vitro* and in lymphocytes) decreases sharply (55). The results correlate with those of Inoue *et al.* (12), who showed that modification of hydroxyl groups, such as that resulting in the formation of syringic acid, abolishes the apoptotic activity of gallic acid. Similarly we have also compared the relative DNA cleavage efficiency of resveratrol, piceatannol, and the parent compound trans-stilbene in plasmid pBR322 DNA (75). It was found that both resveratrol and piceatannol caused conversion of supercoiled plasmid molecules into linear molecules. However, piceatannol also gives rise to smaller-sized heterogeneous fragments as indicated by the formation of a smear on the gel. Thus, piceatannol, having a greater number of hydroxyl groups, is a more efficient DNA cleaving agent than resveratrol. Trans-stilbene, which does not have any hydroxyl group, is not a cleaving agent.

(3) Evidence suggests that the anti-oxidant properties of polyphenolics may not fully account for their chemopreventive effects. For example, it was shown that, although ellagic acid is an anti-oxidant ten times more potent than tannic acid, the latter was more effective in inhibiting skin tumor promotion by 12-O-tetradecanoyl phorbol-13 acetate (TPA) than the former (54). It was suggested that the anti-oxidant effects of these polyphenols might be essential but not sufficient for their anti-tumor activity. In any case, ROS scavenging properties of plant polyphenols may account for their chemopreventive effects but not for any therapeutic action against tumors (71). In this context, it may be noted that several polyphenols have been shown to cause tumor regression in animal models (24,33–35). Expression of the *bcl*-2 proto-oncogene, which blocks apoptosis, decreases cellular production of ROS (76). However, the coadministration of anti-oxidant enzymes, such as superoxide dismutase (SOD) and catalase, prevents curcumin (a plant polyphenol)-mediated apoptosis in human leukemia cells (77). Further, it has been shown that the programmed cell death induced by curcumin in human leukemic T-lymphocytes is independent of the involvement of mitochondria and caspases, suggesting the existence of pathways other than the "classic" ones (78). Caspases are essential for both Fas- and mitochondria-mediated apoptosis. However, inhibition of caspases or the use of cells with defective apoptosis machinery has demonstrated that alternative types of programmed cell death could occur, and such alternative death mechanisms are divided into "apoptosis-like" and "necrosis-like" (79).

(4) Fe3+ and Cu2+ are the most redox-active of the metal ions in living cells. Wolfe *et al.* (80) have proposed that a copper-mediated Fenton reaction, generating site-specific hydroxyl radicals, is capable of inducing apoptosis in thymocytes. In a study with thiol-containing compounds, apoptosis was induced in different cell lines when either free copper or ceruloplasmin (a copper binding protein) was added; however, such activity was not observed when either free iron or the iron-containing serum protein transferrin was added (81). Most of the copper present in human plasma is associated with ceruloplasmin, which has six tightly held copper atoms and a seventh, easily mobilized one (82). In another study supporting these observations, copper was found to enhance the apoptosis-inducing activity of polyphenolic anti-oxidants, whereas iron was inhibitory (83). Although iron is considerably more abundant in biological systems, the major ions in the nucleus are copper and zinc (62). As already mentioned, copper ions occur naturally in chromatin and can be mobilized by metal chelating agents. Burkitt *et al.* (84) suggested that the internucleosomal DNA fragmentation might be caused not only by endonuclease but also by metal-chelating agents, such as 1,10-phenanthroline (OP), which promotes the redox activity of endogenous copper ions and the resulting production of hydroxyl radicals. Thus, the internucleosomal DNA "laddering" often used as an indicator of apoptosis may also reflect DNA fragmentation by non-enzymatic processes. As already mentioned above, several reports indicate that serum (85,86), tissue (87) and intracellular copper levels in cancer cells (88) are significantly increased in various malignancies. Indeed, such levels have been described as a sensitive index of disease activity of several hematologic and non-hematologic malignancies (89).

(5) A comparison of the properties of complexes formed between plant polyphenolics and Cu2+ and Fe3+ should indicate which of these two metal ions could lead to DNA fragmentation in the nucleus when complexed. Not much is known about the properties of such complexes. However, considerable information is available about OP chelation of copper and iron ions. Burkitt *et al.* (84) cited several reasons why Cu2+ rather than Fe3+ may be responsible for OP-stimulated internucleosomal DNA fragmentation in isolated nuclei. For example, the cumulative affinity constants (β3 in 0.1M salt) for chelation of various metal ions by OP are in the order $Cu^{2+} \approx Fe^{2+} > Zn^{2+} > Fe^{3+}$. The complex formed between OP and Cu2+ has a redox potential (E^o for $Cu^{2+}/Cu^{+}=0.17$ V) that favors redox cycling, whereas that for Fe^{3+}/Fe^{2+} is 1.1V, presumably because of stabilization in the ferrous state. Because most polyphenolics are also polycyclic compounds similar in size to OP, conceivably their metal binding properties are also similar.

(6) It has been shown that the polyphenol-mediated apoptotic cell death is closely related to an increase in the concentrations of ROS, possibly generated through the reduction of transition metals in cells (90).

CONCLUSION

The above studies lead to the conclusion that resveratrol is able to cause (i) cellular DNA degradation in mammalian cells, (ii) such DNA breakage is caused by the generation of reactive oxygen species (ROS), and (iii) it involves the mobilization of nuclear copper. In view of the findings in our laboratory (15) and those of others, we suggest that resveratrol, which possesses anti-cancer and apoptosis-inducing activities, is able to mobilize nuclear copper, leading to the formation of ROS, such as the hydroxyl radical, in proximity of the site of DNA cleavage. Essentially, this would be an alternative, non-enzymatic and copper-dependent pathway for the cytotoxic action of resveratrol that is capable of mobilizing and reducing endogenous copper. As such, this would be independent of Fas and mitochondria-mediated programmed cell death. It is conceivable that such a mechanism may also lead to internucleosomal DNA breakage (a hallmark of apoptosis), as internucleosomal spacer DNA would be relatively more susceptible to cleavage by ROS. The generation of ROS in normal cells is under tight homeostatic control (91). However, increased generation of ROS resulting in oxidative stress can be induced by a large number of factors, including metals, drugs and pro-oxidants such as H_20_2, resulting in the induction of apoptosis. Since in various malignancies, copper levels are known to be elevated, presumably the oxidative stress is further enhanced in cancer cells. Most cancer cells have an imbalance in anti-oxidant enzymes compared with normal cells (92). In cancer cells, ROS levels can overwhelm the cells' anti-oxidant capacity, leading to irreversible damage and apoptosis (93). The generation of hydroxyl radicals in the proximity of DNA is well established as a cause of strand scission. It is generally recognized that such reaction with DNA is preceded by the association of a ligand with DNA followed by the formation of hydroxyl radicals at that site. Among oxygen radicals, the hydroxyl radical is most electrophilic with high reactivity and therefore possesses a small diffusion radius. Thus, in order to cleave DNA, it must be produced in the vicinity of DNA (94). This requirement is an essential component of our hypothesis and is also supported by our experimental data. The location of the redox-active metal is of utmost importance because the hydroxyl radical, due to its extreme reactivity, interacts exclusively in the vicinity of the bound metal (95). As already mentioned, copper ions occur naturally in chromatin and can be mobilized by metal chelating agents (84). Further, since cancer cells are known to contain elevated levels of copper (85–88), they may be more subject to electron transfer with polyphenols to generate ROS (60). Thus, because of higher intracellular copper levels in cancer cells, it may be predicted that the cytotoxic concentrations of polyphenols required would be lower in these cells as compared to normal cells. Indeed, some plant polyphenols, including resveratrol, have shown such a differential cytotoxic effect for certain cancer cell lines as compared to normal cells (20,96,97). For example, in the case of EGCG, IC_{50} for SV40 virally transformed cells was 10 μM *versus* 120 μM for normal cells (96).

Further, the question of bioavailability of resveratrol in mammalian systems also needs to be addressed. Evidence suggests that polyphenolic compounds, such as tannins and resveratrol, are able to traverse cell membranes and may enter the cytoplasmic or nuclear space. Resveratrol is sufficiently hydrophobic and has been shown to be present in tissues such as heart, liver and kidney (98). However, Asensi *et al.* (99) reported that resveratrol may have a relatively low bioavailability due to its biotransformation and rapid elimination. It was shown that the highest concentration of resveratrol in plasma was reached within the first 5 min (2.6 ± 1 μM) after receiving 20 mg res/kg.b. w orally (99). Nevertheless, these authors further report that 5 μM resveratrol completely inhibited the growth of B-16 M murine melanoma cells. It is to be noted that in our studies, the minimum concentration of resveratrol used (such as 10 μM in Fig. 4) is able to induce measurable cellular DNA cleavage and thus could be physiologically relevant. Further, in real life human situation, a sustained

intake of resveratrol in the form of beverages such as red wine and fruits and nuts such as grapes and peanuts, etc. may maintain the required physiologically relevant intracellular concentration cytotoxic to neoplastic cells. In addition, it is well established that a combination of various polyphenols is considerably more effective in cytotoxicity towards cancer cells than individual polyphenols alone (100). Studies in our laboratory have further shown that properties which form the basis of the above-described cytotoxic mechanism, such as DNA binding, Cu(II) reduction, mobilization of endogenous copper and generation of ROS, are common to several classes of polyphenols (55–59,63,64,67,69,75). We believe that such a common mechanism better explains the anti-cancer effects of polyphenols with diverse chemical structures as also the preferential cytotoxicity towards cancer cells.

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