JOURNAL OF MEDICINAL FOOD
J Med Food 16 (3) 2013, 199–205

DOI: 10.1089/jmf.2012.0170

Use of Grape Polyphenols Against Carcinogenesis: Putative Molecular Mechanisms of Action Using *In Vitro* and *In Vivo* Test Systems

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ABSTRACT Polyphenols are present in foods and beverages and are related to sensorial qualities such as color, bitterness, and astringency, which are relevant in wine, tea, grape juice, and other products. These compounds occur naturally in forms varying from simple phenolic acids to complex polymerized tannins. Thus, it is reasonable to expect that grape-derived products elaborated in the presence of skins and seeds, such as wine and grape juice, are natural sources of flavonoids in the diet. Carcinogenesis is a multistep process that is characterized by genetic, epigenetic, and phenotypic changes. With increasing knowledge of these mechanisms, and the conclusion that most cases of cancer are preventable, efforts have focused on identifying the agents with potential anticancer properties. The use of grape polyphenols against the carcinogenesis process seems to be a suitable alternative for either prevention and/or therapeutic purposes. The aim of this article is to show the molecular data generated from the use of grape polyphenols against carcinogenesis using *in vivo* and *in vitro* test systems.

KEY WORDS: • ***cancer*** • ***carcinogenesis*** • ***grape polyphenol***

INTRODUCTION

CARCINOGENESIS IS A MULTISTEP PROCESS that is characterized by genetic, epigenetic, and phenotypic changes.[1] Such changes involve genetic damage; mutation in critical genes related to the control of cell division, cell death, and metastatic potential; and activation of signalizing or metabolic pathways that give the cells favorable growth and survival characteristics.[2] With increasing knowledge of these mechanisms, and the conclusion that most cases of cancer are preventable, efforts have focused on identifying the agents with potential anticancer.[3]

Polyphenols comprise a group of substances with different chemical structures, with only a few present in the human diet. Initially, polyphenols act as an antioxidant defense of plants, preventing the oxidative damage caused by ultraviolet rays.[4] In foods and beverages, these compounds are related to sensorial qualities, such as color, bitterness, and astringency in wine, tea, grape juice, and other products.[5–8] Polyphenols occur in a variety of forms, from simple phenolic acids to complex polymerized tannins. Fruits and derived products, such as grape juices, pulps, and jams, are among the main sources of polyphenols in the diet.[9] According to their chemical structure, polyphenols of nutritional interest are commonly divided into three groups: phenolic acids, flavonoids, and stilbens.[10,11] Due to the substances' high instability to light and oxygen, the compounds undergo transformations during processing, storage, and extraction procedures. Hence, estimates of their content in foods and of the human intake are difficult to predict. Yilmaz and Toledo[12] have postulated that flavonoids are abundant in grape seeds and skin, showing important antioxidant capacity. Thus, it is reasonable to expect that grape-derived products elaborated in the presence of skins and seeds, such as wine and grape juice, are natural sources of flavonoids in the diet. Anthocyanins, flavan-3-ols, and oligomeric procyanidins are the flavonoids present in important amounts.[13,14]

Although considered a powerful antioxidant, recently, new mechanisms for polyphenols' physiological effects have been proposed. For example: modulation of gene expression, induction of apoptosis, decrease in platelet aggregation, increase in blood vessel dilation, modulation of intercellular signaling, modulation of enzyme activities associated with carcinogen activation and detoxification, and chelation of transition metals such as iron.[15,16] Herein, the aim of this article is to show current molecular data generated from the use of grape polyphenols in carcinogenesis studies using *in vitro* and *in vivo* test systems.

METHODOLOGY

A comprehensive literature search for studies on grape polyphenol, cancer, and/or carcinogenesis was performed.

Manuscript received 3 July 2012. Revision accepted 30 October 2012.

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In brief, a search of PubMed, MEDLINE, Embase, and Google Scholar for a variety of articles (all publications ranging from 2007 until May 2012) was carried out. Case reports were excluded from the review. Abstracts were reviewed and relevant articles were identified.

RESULTS

In vitro *studies*

Resveratrol is a nonflavonoid polyphenol compound present in many plants and fruits, and is found at especially high concentrations in the grape. Some authors have postulated that pretreatment with a p38 inhibitor enhanced resveratrol-induced apoptosis by accelerating the intrinsic apoptotic pathway, including Bax activation, loss of mitochondrial membrane potential, and activation of both caspase-9 and -3 in human lung adenocarcima cells.[17] Although treatment with resveratrol alone did not induce caspase-8 activation, co-treatment with both p38 inhibitor and resveratrol not only enhanced FasL cleavage but also activated caspase-8, indicating that the extrinsic apoptotic pathway may be involved in the synergistic effect.[17] Resveratrol treatment was also able to induce the activation of Bak but not Bax, and silencing Bak but not Bax by shRNA almost completely prevented resveratrol-induced cell death, mitochondrial dysfunction, and also largely prevented resveratrol-induced apoptosis inducing factor release, demonstrating the preferential engagement of Bak but not Bax during resveratrol-induced apoptosis in both cell lines.[18] In addition, resveratrol treatment induced a significant degradation of induced myeloid leukemia cell differentiation protein (Mcl-1), while knockdown of Mcl-1 by shRNA only modestly increased resveratrol-induced Bak activation. Interestingly, silencing Bim but not Puma and Noxa remarkably attenuated resveratrol-induced cell death, loss of mitochondrial membrane potential, and Bak activation, suggesting the important roles of Bim.[18] The treatment with resveratrol increased the levels of activated checkpoint kinase 2 expression (p-Chk2), which showed a decrease of cyclin A expression.[19] While the levels of cyclin dependent kinase 2 (CDK2) remained unchanged by treatments, its active form [Thr(160)-phosphorylated CDK2] was decreased by treatment with resveratrol.[19] The activity of CDK7, the kinase that phosphorylates CDK2 at Thr(160), was inhibited by resveratrol.[19]

Although the efficacy on the apoptosis process has been documented, the bioefficacy is yet a matter of debate. For this reason, resveratrol was compared with its derivatives, triacetyl-resveratrol (trans-3,5,4′-triacetylstilbene) and trimethoxy-resveratrol (trans-3,5,4′-trimethoxystilbene) in both estrogen receptor-α (ERα)-positive and ERα-negative breast cancer cells. Binding to integrin $\alpha v\beta 3$ and control of cell proliferation and p53 were chosen as targets for comparative analysis using an *in silico* and biochemical approach.[20] Resveratrol and triacetyl-resveratrol interacted avidly and specifically with integrin $\alpha v\beta 3$ through binding at the site targeted by the high affinity cyclic Arg-Gly-Asp (RGD) peptide. In contrast, binding of trimethoxy-resveratrol to this site was substantially less robust. Moreover, the different stilbenes also elicited diverse cellular and signaling responses in target cells.[20] Further, stilbene-elicited signaling cascade leading to p53 activation was examined and results showed that resveratrol and triacetyl-resveratrol induced both ERK and p38 phosphorylation, whereas only marginal changes in state of phosphorylation in these two kinases were observed in trimethoxy-resveratrol-treated cells.[20]

Estrogen plays a crucial role in the development of breast cancer, and the inhibition of estrogen synthesis has been an important target for the prevention and treatment. As a result of its structural resemblance to estrogen, resveratrol's agonistic and antagonistic properties on ER have been examined. Resveratrol inhibited the aromatase activity with a half-maximal inhibitory concentration (IC_{50}) of 25 μM on ERα-positive cells. Kinetic analysis indicated that both competitive and noncompetitive inhibition might be involved.[21] The cell proliferation specifically induced by testosterone was significantly reduced by 10 μM resveratrol. In addition, 50 μM resveratrol significantly reduced the CYP19-encoding mRNA abundance.[21] Luciferase reporter gene assays revealed that resveratrol could repress the transcriptional control dictated by the promoter regulation.[21]

In human fibrosarcoma cells, resveratrol was able to block adherence to endothelial cells in a dose-dependent manner.[22] To further elucidate the mechanisms of this resveratrol-mediated blockade of tumor cell adhesion, the expression of the cell adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, and E-selectin were examined.[22] Resveratrol reduced the ICAM-1 expression. Taking into consideration that the induction of ICAM-1 requires activation of the transcription factor nuclear factor kappa-B (NFκB), the effects of resveratrol on the activation of this factor was also investigated. Resveratrol inhibited the NFκB activation and NFκB-dependent luciferase activity.[22] After resveratrol treatment, a strong inhibition of tumoral cardiac cell growth was associated with a loss of cell adhesion.[23] Cell proliferation arrest was associated with an apoptotic process revealed by an increased percentage of cells with fragmented and/or condensed nuclei, which undergoes apoptosis. By contrast, no cytotoxic effects of resveratrol were observed, and a protective outcome of resveratrol against norepinephrine-induced apoptosis was found on normal cardiomyocytes.[23]

Epidemiological studies suggested that trans-resveratrol, a wine grape component, could prevent malignant tumor development. This compound also demonstrated cytostatic and cytotoxic effects on tumor cells *in vitro*. Some authors have suggested that resveratrol triacetate and vineatrol are efficient in inducing the accumulation of human colon cancer cells in the early S phase of the cell cycle.[24] This effect is associated with a nuclear redistribution of cyclin A and the formation of a cyclin A/cyclin-dependent kinase 2 complex whose kinase activity is increased.[24] Of particular importance, resveratrol triacetate and vineatrol dramatically enhance 5-fluoro-uracil–mediated inhibition of colon cancer cell proliferation.[24] The potential inhibitory effect of the resveratrol derivative on cell proliferation and the

underlying mechanisms of this effect on human pancreatic cancer cell lines were evaluated. After 7 days of incubation, resveratrol derivative inhibited the growth of cells with IC_{50} values of 9.6 and 8.7 μM, respectively. Resveratrol derivative (40 μM) arrested cells in the G0/G1 phase and depleted cells in the S phase of the cell cycle (-105% and -35% of control, respectively). Resveratrol derivative induced dose-dependent apoptosis in both pancreatic cancer cell lines and was found to significantly reduce the *in situ* activity of ribonucleotide reductase, the key enzyme of DNA synthesis. Employing growth inhibition assays, K resveratrol derivative acted synergistically with gemcitabine in both cell lines.[25]

Several studies have documented the anticancer and chemopreventive efficacy of grape seed extract against various malignancies, including prostate cancer. Grape seed extract is a complex mixture of polyphenols, including gallic acid, catechin, epicatechin, and procyanidins-oligomers of catechin and epicatechin, some of which are esterified with gallic acid. The most active procyanidin was identified by mass spectrometry and enzymatic hydrolysis as the 3,3′-di-O-gallate ester of procyanidin dimer B2 (Epi-Epi). B2-digallate exhibited dose-dependent effects on prostate cancer cells over the range 25–100 μM, whereas gallic acid exhibited comparable activity at lower doses but was highly lethal at 100 μM.[26] These data, and the fact that nonesterified B2 exhibited little or no activity, suggest that the galloyl groups of B2-digallate are primarily responsible for its effects on prostate cancer cells cells.[26]

When the activity of grape seed extract on vascular endothelial growth factor (VEGF) receptor and angiogenesis was investigated, the xenobiotic inhibited the kinase activity of purified VEGF receptor.[27] Grape seed extract could also inhibit the VEGF receptor/mitogen-activated protein kinase-mediated signaling pathway in endothelial cells.[27] As a result, grape seed extract could inhibit VEGF-induced endothelial cell proliferation and migration as well as sprout formation from aorta ring.[27] Some authors have demonstrated that grape seed extract inhibited VEGF messenger RNA (mRNA) and protein expression in human glioma cells and human breast cancer cells.[28] Moreover, grape seed extract inhibited transcriptional activation of the VEGF gene through reducing protein but not mRNA expression of hypoxia-inducible factor (HIF)–1α.[28] The inhibitory effect of grape seed extract on HIF-1α expression was mainly through inhibiting HIF-1α protein synthesis rather than promoting protein degradation. Consistent with this finding, grape seed extract-suppressed phosphorylation of several important components involved in HIF-1α protein synthesis, such as Akt, S6 kinase, and S6 protein. Grape seed extract inhibited the expression of VEGF and HIF-1α and the phosphorylation of S6 kinase without altering the subcellular localization of HIF-1α, correlating with reduced vessel density and tumor size.[28] Depletion of polyphenol with polyvinylpyrrolidone abolished the inhibitory activity of grape seed extract, suggesting a water-soluble fraction of polyphenol in grape seed extract is responsible for the inhibitory activity.[28]

Grape extract also presents a strong antiradical activity in the *in vitro* 2,2-diphenyl-1-picrylhydrazyl radical assay and protects against reactive oxygen species production in human colon adenocarcinoma cells.[29] In contrast, the extract did not protect in the citronellal thermooxidation system and showed a weak protective action against lipid peroxidation in colon adenocarcinoma cells.[29] The clonogenic assay and the cell cycle distribution analysis showed that the grape extract has a significant antiproliferative effect in a tumor cell line.[29] Polyphenol-rich berry extracts were screened for their antiproliferative effectiveness using human cervical cancer cells grown in microtiter plates. Rowan berry, raspberry, lingonberry, cloudberry, arctic bramble, and strawberry extracts were effective but blueberry, sea buckthorn, and pomegranate extracts were considerably less effective. The most effective extracts (strawberry > arctic bramble > cloudberry > lingonberry > raspberry > rowan berry) gave half-maximal effective concentration (EC_{50}) values in the range of 25–40 μg of extract/mL of phenols. These extracts were also effective against human colon cancer cells, which were generally more sensitive at low concentrations but conversely less sensitive at higher concentrations.[30] The strawberry, cloudberry, arctic bramble, and raspberry extracts share common polyphenol constituents, especially the ellagitannins, which have been shown to be effective antiproliferative agents. Therefore, the antiproliferative activity of lingonberry was caused predominantly by procyanidins.[30]

There is considerable interest in alternative/adjuvant approaches for the eradication of *Helicobacter pylori* using biologically active compounds, especially antioxidants from plants. Hydroalcoholic extracts from Colorino, Sangiovese, and Cabernet Sauvignon grape cultivars against *H. pylori* G21 (cagA-negative, cagA−) and 10K (cagA-positive, cagA+) clinical isolates were tested. The Colorino extract showed the highest antibacterial activity against the G21 strain (MBC = 1.35 mg/mL), while Sangiovese and Carbernet MBCs were $\sim$4.0 mg/mL. *H. pylori* 10K was only susceptible to Colorino after 48 h (MBC = 3.57 mg/mL).[31] Resveratrol exhibited the highest antibacterial activity. Interestingly, the most pathogenic strain (10K) was less susceptible to both the grape extracts and the isolated compounds.[31] Moreover, resveratrol shows an evident toxic activity at high concentrations. Conversely, the xenobiotic can effectively inhibit the synthesis of polyomavirus DNA at subcytotoxic concentrations.[32] The antiviral action is evident after the phase of virion entry; therefore, data suggest that the drug acts during the synthesis of the viral progeny DNA.[33] Flow cytometric analysis of Annexin-V labeled cells indicated that Cretan Sultana, Nemea, and Messinia currants at 500 μg dried product/mL medium significantly induced cell death. All extracts from 500 μg dried raisins statistically decreased protein and mRNA levels of ICAM-1 in tumor necrosis factor (TNF)-α fibroblast cells. Measurement of IL-8 protein levels and quantification for IL-8 mRNA showed no significant decrease.[33] These results indicate that the methanol extracts from currants, rich in phenolic compounds, exhibit cancer preventive efficacy by limiting cell proliferation, inducing cell death, and suppressing ICAM-1 levels.

Anthocyanins are polyphenol antioxidants that have been shown to prevent many chronic diseases, including cancer. The compounds are largely metabolized by various enzymes and bacteria in the large intestine, and the health benefits of consuming foods rich in anthocyanins could be due mostly to the effects of these metabolites. An anthocyanin extract from Cabernet Sauvignon grapes that contained delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside was employed.[32] The extract was incubated anaerobically in the contents of the large intestine of freshly slaughtered pigs for 0, 0.5, and 6 h (final concentrations of 20.9, 28.2, 61.4, and 298.0 μM of the above anthocyanin compounds, respectively, at $t = 0$ h). After 6 h, anthocyanins were no longer detected, and three metabolites were identified as 3-O-methylgallic acid, syringic acid, and 2,4,6-trihydroxybenzaldehyde.[32] Results from this study suggest that consumption of Cabernet Sauvignon grape anthocyanins could lead to the formation of specific metabolites in the human gut, and it is possible that these metabolites offer the protective effect against colon cancer attributed to anthocyanin consumption.[32]

Piceatannol (trans-3,4,3′,5′-tetrahydroxystilbene) is a polyphenol that is found in grapes, red wine, *Rheum undulatum*, and the seeds of *Euphorbia lagascae*. Piceatannol reduced the viable numbers and increased the numbers of apoptotic prostate cancer cells in a dose-dependent manner. Western blot analysis revealed that piceatannol increased the protein levels of cleaved caspase-8, -9, -7, and -3 and cleaved poly (ADPribose) polymerase (PARP).[34] Piceatannol increased mitochondrial membrane permeability and cytochrome c release from the mitochondria to the cytosol.[34] Also, piceatannol induced an increase in the levels of truncated Bid, Bax, Bik, Bok, and Fas but caused a decrease in the levels of Mcl-1 and Bcl-xL. Caspase-8 and -9 inhibitors mitigated piceatannol-induced apoptosis. The caspase-8 inhibitor suppressed the piceatannol-induced cleavage of Bid, caspase-3, and PARP.[34] Piceatannol inhibited the proliferation of T24 and HT1376 human bladder cancer cells by blocking cell cycle progression in the G0/G1 phase and inducing apoptosis. ELISA showed that the G0/G1 phase arrest is due to an increase in the expression of p21/WAF1. An enhancement in Fas/APO-1 and membrane-bound Fas ligand (mFasL) might be responsible for the apoptotic effect induced by piceatannol.[35]

Freeze-dried grape powder (FDGP) treatment on primary hepatocytes and hepatoma cells revealed increased metabolic activity of cells and phosphorylation of Akt and IκBα, as well as upregulation of proliferating cell nuclear antigen level.[36] To further elucidate the molecular mechanisms of the complex mixture, cells were treated with TNF-related apoptosis-inducing ligand (TRAIL); taurodeoxycholic acid (TDCA); thapsigargin (TG), to induce cell apoptosis through the death receptor–, mitochondria-, or ER-mediated pathway; and H_2O_2, to induce oxidative stress. TDCA-induced activation of caspase-3, caspase-7, caspase-9, and Bax was dramatically decreased. Furthermore, FDGP reduced levels of annexin V–positive cells four-fold. Also, FDGP pretreatment restored cellular glutathione content by 71% in cells treated with H_2O_2.[36]

In vivo *studies*

To the best of our knowledge, there are few studies investigating grape polyphenols against carcinogenesis, especially in humans. Comparative evaluation of antitumor-promoting effects of seedless and seeded grape polyphenolic extracts was carried out in carcinogen-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)–promoted S/RVCriba mouse skin and stomach, as well as esophagus of mice. Both seedless and seeded grape polyphenolic extracts possessed antitumor-promoting activity in target tissues of mice as is evident from their ability to delay tumor formation along with a significant decrease in tumor multiplicity and incidence.[37] Marked and sustained epidermal hyperplasia observed in 7,12-dimethylbenz(a)anthracene–initiated and TPA-promoted mice was greatly reduced on pretreatment with grape polyphenolic extracts or catechin.[37] The polyphenolic extracts from Sharad seedless and seeds of Bangalore blue showed the strongest suppressing activity comparable to catechin than the corresponding whole grapes.[37] The inclusion of dietary grape antioxidant dietary fiber induced alterations in the expression of tumor suppressor genes and proto-oncogenes as well as the modulation of genes from pathways, including lipid biosynthesis, energy metabolism, cell cycle, and apoptosis. Overexpression of enzymes pertaining to the xenobiotic detoxifying system and endogenous antioxidant cell defenses was also observed.[38]

BALB/c mice were subcutaneously implanted with C26 colon carcinoma cells, and 2 days later they received either solvent or red wine polyphenols (RWPs; 100 mg/kg per day, human equivalent dose ~500 mg/day) in the drinking water for 25 days. Wistar rats received either solvent or RWPs (100 mg/kg per day, human equivalent dose approximately 1000 mg/day) in the drinking water 1 week before injection of azoxymethane and were studied 10 weeks later.[39] In mice, RWPs inhibited tumor growth by 31%, reduced tumor vascularization and the number of lung metastases, decreased proliferation as indicated by downregulation of Ki67, cyclin D1, and increased apoptosis as indicated by TUNEL staining and active caspase-3 levels in tumor cells.[39] RWPs reduced expression of VEGF, matrix metalloproteinase (MMP)-2, MMP-9, and cyclooxygenase-2, and increased expression of tumor suppressor genes *p16* (*INK4A*), *p53*, and *p73* in tumor cells. In rats, RWPs reduced by 49% the number of azoxymethane-induced aberrant crypt foci (preneoplastic lesions) in the colon.[39]

The combined effect of dietary grape polyphenols (5 mg/kg each of resveratrol, quercetin, and catechin) was tested on progression of mammary tumors in nude mice created from green fluorescent protein-tagged MDA-MB-435 bone metastatic variant. Fluorescence image analysis of primary tumor growth demonstrated a statistically significant decrease in tumor area by dietary grape polyphenols.[40] Molecular analysis of excised tumors demonstrated that reduced mammary tumor growth may be due to upregulation of FOXO1 (forkhead box O1) and NFKBIA (IκBα); thus, activating apoptosis and potentially inhibiting NFκB activity.

Image analysis of distant organs for metastases demonstrated that grape polyphenols reduced metastasis, especially to liver and bone.[40]

Resveratrol was able diminish the severity of esophagitis, incidence of intestinal metaplasia, and incidence of carcinoma as compared with both the saline and nonoperated control groups in a rat established model.[41] Although resveratrol has been found to exhibit chemopreventive actions in experimentally induced skin, breast, colon, and esophagus rodent tumors, chemopreventive potential of this dietary constituent has not been explored well against experimental liver cancer. The inhibitory effect of resveratrol using a two-stage model of rat hepatocarcinogenesis in Sprague-Dawley rats was performed. Initiation was performed by a single intraperitoneal injection of diethylnitrosamine (DENA; 200 mg/kg), followed by promotion with phenobarbital (0.05%) in drinking water. Resveratrol dose-dependently reduced the incidence, total number and multiplicity of visible hepatocyte nodules.[42] Mean nodular volume and nodular volume as percentage of liver volume were also inhibited upon resveratrol treatment.[42] Immunohistochemical detection of cell proliferation and assay of apoptosis indicated a decrease in cell proliferation and increase of apoptotic cells in the livers of resveratrol-supplemented rats. Resveratrol also induced the expression of proapoptotic protein Bax, reduced antiapoptotic Bcl-2 expression, with a concurrent increase in Bax/Bcl-2 ratio.[42]

The antitumor properties of the Merlot grape (and Merlot wine) polyphenols were evaluated in relation to their ability to modulate gene expression in developing tumors using an athymic nude mouse model transplanted with the ER-negative cells. The development of tumors was almost totally arrested in grape polyphenol–treated mice. Total polyphenols isolated from the wine were more effective in reducing tumor growth as compared with a hydrophobic polyphenol fraction isolated from the wine.[43] Analysis of gene expression showed that genes, such as *CDK2*, *FAS*, *LEF1*, *PRKCE*, and *PTGS2*, belonging to the NFκB, phospholipase C, and calcium signaling pathways, were downregulated in tumors that developed in grape polyphenol–treated mice.[43] Several genes related to cell cycle regulation, such as *CDK5RAP1*, *RBBP8*, and *SERTAD1*, were upregulated in these tumors.[43]

Some authors have tested the effect of low dietary concentrations of resveratrol, quercetin, and catechin on breast cancer progression. The effects of these compounds on fluorescently tagged breast tumor growth in nude mice were assessed using *in situ* fluorescence image analysis. Individual polyphenols at 0.5 μM neither decreased breast cancer cell proliferation nor affected cell cycle progression *in vitro*. Furthermore, using *in situ* image analysis, we determined that combined dietary polyphenols at 0.5, 5, or 25 mg/kg reduced primary tumor growth of breast cancer xenografts in a nude mouse model.[44]

In another study, 34 female patients with histologically confirmed breast cancer, stages IIIB and IV were enrolled in a randomized controlled trial. The Physical Well-Being subscale score of the QOL FACT-B version 4 questionnaire showed a significant difference between the two groups. Breast Cancer Score (Additional Concerns) had a borderline significant difference. The Social/Family Well-Being subscale and Emotional Well-Being subscale scores showed no significant difference. At inclusion, radical activity >310 FORT units, relevant for increased oxidative stress, were present in 95.1% cases. After 3 months, radical activity >310 FORT units were present in 52.8% cases in group 1.[45] *In vivo* assay further showed that grape seed extract could inhibit tumor growth and tumor angiogenesis of breast cancer cells in mice.[27] Consistent with the *in vitro* data, grape seed extract treatment of tumor-bearing mice led to concomitant reduction of blood vessel density and phosphorylation of mitogen-activated protein kinase.[27]

CONCLUSION

In this article, we have showed recent studies focusing on the putative molecular mechanisms exerted by grape polyphenols against carcinogenesis using *in vitro* and *in vivo* test systems. Although these data have revealed important biomarkers for understanding the mechanisms of action induced by grape polyphenol on eukaryotic cells, either ordinary or tumor cells, much remains to be examined. More adequately powered, randomized, placebo-controlled human studies, as well as animal studies are needed on polyphenols for better understanding the role of this xenobiotic. Therefore, this area warrants further investigation as a new way of thinking, which would apply not only to polyphenols but also to other phytochemicals used as promising therapeutic agents against oral human diseases. Regardless, this study reveals grape polyphenols as a nutraceutical agent against carcinogenesis.

ACKNOWLEDGMENTS

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). D.A.R. is a recipient of the CNPq fellowship.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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