ELSEVIER

Clinical Biochemistry 39 (2006) 492–497

CLINICAL BIOCHEMISTRY

A comparison of the anticarcinogenic properties of four red wine polyphenols

George J. Soleas[a,b], Linda Grass[c], P. David Josephy[d], David M. Goldberg[b] Eleftherios P. Diamandis*[,b,c]

[a]*Quality Assurance Department, Liquor Control Board of Ontario, 55 Lakeshore Blvd. E., Toronto, Ontario M5E 1A4 Canada*
[b]*Department of Laboratory Medicine and Pathobiology, University of Toronto, 100 College Street, Toronto, Ontario M5G 1L5, Canada*
[c]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada*
[d]*Department of Chemistry & Biochemistry, University of Guelph, Ontario, Canada*

Received 13 November 2001; received in revised form 16 January 2002; accepted 25 January 2002

Abstract

Background: There has been growing interest in the analysis of certain polyphenols in wine, especially flavonoids, trihydroxystilbenes and phenolic acids, stimulated by intense research into their potential benefits to human health. One of their main properties in this regard is their antioxidant activity, which enables them to attenuate the development of atherosclerosis, inflammatory diseases, and cancer.
Methods: A two stage CD-1 mouse skin cancer model using 9,10-dimethyl-1,2-benzanthracene (DMBA) as initiator and phorbol 12-myristate 13-acetate (TPA) as promoter was employed to compare the antitumorigenic activities of one polyphenol from each of four different classes: flavanols [(+)-catechin], stilbenes (*trans*-resveratrol), flavonols (quercetin) and hydroxybenzoic acids (gallic acid). Animals were treated with specific polyphenols at doses ranging from 0 to 25 μmoles (dissolved in 200 μL acetone), twice a week for eighteen weeks. The solution was applied topically to the shaved dorsal region of each animal. The relative potencies of the polyphenols were compared by evaluating the percentage inhibition of tumor formation in individual mice and the number of mice developing one or more tumors with the different dose schedules.
Results: Probit analysis revealed that quercetin was the most (ED_{50}<1 μmole) and gallic acid the least effective (ED_{50} 5–10 μmoles). (+)-Catechin and *trans*-resveratrol were intermediate, with ED_{50} values of 5 and 6 μmoles, respectively.
Conclusion: We have shown recently that *trans*-resveratrol is absorbed much more efficiently than (+)-catechin and quercetin in humans after oral consumption. Taking this and the relative concentrations in red wine into account, together with the present results, we conclude that *trans*-resveratrol may be the most effective anticancer polyphenol present in red wine as consumed po by healthy human subjects.

1. Introduction

A large body of literature has been devoted to studies describing the potential anticancer activities of red wine polyphenols [1–3]. In many instances, these effects can be attributed to plausible biochemical mechanisms including enhanced apoptosis, growth arrest at one or more points in the cell cycle, inhibition of DNA synthesis, and modulation of signal transduction pathways by altered expression of key enzymes such as cyclooxygenases and protein kinases. Many experimental approaches have been used in these investigations, including use of cell lines, whole animals and, in a few instances, human cancer patients. Four of these compounds have attracted particular attention.

1.1. Quercetin

This flavone 3-ol (2-[3,4-dihydroxyphenyl]-3,5,7-trihydroxy-4H-1-benzopyran-4-one) has a wide spectrum of anticancer properties including inhibition of the growth of cells derived from human cancers such as those of stomach [4], colon [5,6], prostate [7] and breast [8]. Additionally, it suppresses the growth and development of uterine cervical cancer [9], melanomas [10], and intestinal tumors [11] in whole mice. In a phase I clinical trial, quercetin administered by IV (IV) infusion lowered by sixfold the serum concentration of CA125, a protein marker for ovarian cancer, in a terminal patient with this disease; in another patient

* Corresponding author. Tel.: +1-416-586-8443; fax: +1-416-856-8628.
E-mail address: ediamandis@mtsinai.on.ca (E.P. Diamandis).

0009-9120/02/$ – see front matter © 2002 The Canadian Society of Clinical Chemists. All rights reserved.
doi:10.1016/j.clinbiochem.2006.04.014

Reprinted from Volume 35:119–24, 2002.

with advanced hepatoma, serum α-fetoprotein (a marker correlating with hepatic tumor burden) was significantly diminished [12]. However, the same authors subsequently found that a water-soluble pro-form of quercetin was ineffective when given po because of poor absorption [13].

1.2. (+)-Catechin

This is a flavane 3-ol (2-[3,4-dihydroxyphenyl]-3,4-dihydro-2H-1-benzopyran-3,5,7-triol). Abundantly present in fruits and vegetables, it and a family of related congeners are the principal polyphenols of green tea which is gaining recognition as a beverage with considerable anticancer potential. (+)-Catechin is effective in blocking the growth of human cell lines originating from cancers of the prostate [7,14] and breast [8], as well as inhibiting tobacco-induced carcinogenesis in rat hepatocytes [15]. Its ability to prevent cancer initiation receives strong support from a recent investigation in which it suppressed by 75% the occurrence of intestinal tumors in mice bearing a germline defect causing these lesions to arise spontaneously [16].

1.3. Trans-Resveratrol

This trihydroxy-stilbene is present in a number of plants, but in few sources of human nutrition. Nuts, grapes, and red wine are the principal examples. In 1997, it was reported that *trans*-resveratrol (1–25 μM) inhibited the initiation and promotion of hydrocarbon-induced skin cancer in mice, as well as the progression of breast cancer in this species [17]. Potent antimutagenic activity was also demonstrated [18]. These observations have been extended by a multiplicity of reports confirming by means of *in vitro* and whole animal models that *trans*-reseveratrol is a very potent and versatile anticancer agent targeting several different sites in the neoplastic process.

Its anticancer activity against human neoplasms has been shown by many investigations utilizing human cancer cell-lines, including those from breast [8,9,20], prostate [7,21], colon [22,23] and oral squamous carcinoma [24]. One mechanism responsible for this behavior seems to be apoptosis [26–28], a final common pathway that can be initiated by the action of *trans*-resveratrol on various signal pathways regulating the cell cycle, including modulation of tumor suppressor genes. A different mechanism that may be important in the prevention of chemical carcinogenesis by *trans*-resveratrol implicates the aryl hydrocarbon receptor system necessary for the uptake and activation of many carcinogens; these processes are blocked by *trans*-resveratrol at very low concentrations (<1 μM), especially in whole animal experiments [29,30]. Recently, *trans*-resveratrol has been shown to inhibit the metastasis of primary tumors in mice and their ability to induce the proliferation of blood vessels into the tumor tissue, thus depriving the malignant cells of oxygen and nutrients [31].

1.4. Gallic acid

This hydroxybenzoic acid is present in many fruits and vegetables, but, like (+)-catechin and its congeners with which it forms a family of esters, the most important nutritional source of the compound is green tea. It is also used as an antioxidant food additive, *e.g.*, lauryl gallate and other alkyl esters, agents that demonstrate the ability to kill animal tumor cells by inducing apoptosis [32,33]. The parent compound manifests similar effects on human cancer cells derived from lung [34], stomach and colon [35], as well as human leukemic cells [36].

As a constituent of green tea, gallate derivatives are able to promote signal-induced growth arrest of human breast cancer cells [37], to inhibit hydrocarbon-induced mutagenesis in mice [38], to induce apoptosis in human peripheral blood lymphocytes [39] and in human prostate cancer cells [40].

Most of the reports cited above are based upon experiments in which one compound (or one family of compounds) was tested. Moreover, the vast majority are based upon attempts to prevent growth or promote death of established solid cancers or cancer cell-lines. For various reasons, to be discussed later, it is very unlikely that red wine polyphenols will be adequately absorbed and reach the sustainable effective concentrations necessary for the successful treatment of human cancers. A more plausible role would be in preventing cancer. Optimal prophylaxis would almost certainly require dietary supplementation, since in most Western countries other than those in the Mediterranean basin, fresh fruit and vegetables as well as wine do not figure prominently in the general diet, especially among the lower socio-economic classes where cancer risk is usually greatest. However, there is little comparative information about the relative efficacy of the four compounds of interest in cancer prevention. This report describes our attempt using a two-stage mouse skin cancer model to compare their antitumorigenic activities.

2. Materials and methods

2.1. Reagents & chemicals

Dimethyl sulfoxide (DMSO) and acetone all distilled in glass were purchased from Mallinckrodt-Baker, Phillipsberg, New Jersey. Phorbol 12-myristate 13-acetate (TPA; cat. no. P 8139) and 9,10-dimethyl-1,2-benzathracene (DMBA; cat. no. D 3254), (+)-catechin (cat. no. 86181–2), quercetin (cat. no. 17196−4), *trans*-resveratrol (cat. no. R5010) and gallic acid (cat. no. R 8647) were all purchased from Sigma-Aldrich Canada Ltd., Oakville, Ontario. Stock TPA was prepared by dissolving 25 mg in 1 mL of DMSO. This product was stable in solution for six months. Stock DMBA was prepared by dissolving 6.4 mg in 25 mL of

acetone. All four polyphenols were dissolved in acetone individually to achieve the desired concentrations.

2.2. Methods

Six groups of five CD-1 mice were used to evaluate the antitumorigenicity of each of the four compounds. They were purchased from Charles River Corporation (Quebec, Canada) and were housed separately in disposable cages. They were fed chow and tap water *ad lib.* Five of the six groups were initially treated with DMBA at a dose of 200 nmoles in 200 μL acetone. The solution was applied topically to the shaved dorsal region of each animal. The sixth group was used as a negative control receiving only acetone initially and biweekly. The animals were treated topically thereafter with one of the 4 phytochemicals at doses of 0 (positive control), 1, 5, 10, and 25 μmoles dissolved in 200 μL acetone along with 5 nmoles of TPA twice a week. This process continued for eighteen weeks. The flanks were shaved weekly to observe tumor development that usually commenced between weeks 8 to 10. When tumors became numerous, shaving had to be discontinued to prevent infection. The tumors were counted at the end of the eighteenth week when the skin was carefully shaved to fully reveal all the lesions. The mean of each group and % reduction of tumorigenicity relative to the positive controls were calculated and plotted against the dose.

The full protocol for this study was approved by the Animal Experimentation committee of the University of Toronto. It must be emphasized that the use of rubber gloves is at all times essential because of the risk to personnel in handling animals bearing chemical carcinogens. All experiments were carried out at the Animal Facility of Mount Sinai Hospital, Toronto, Ontario, Canada.

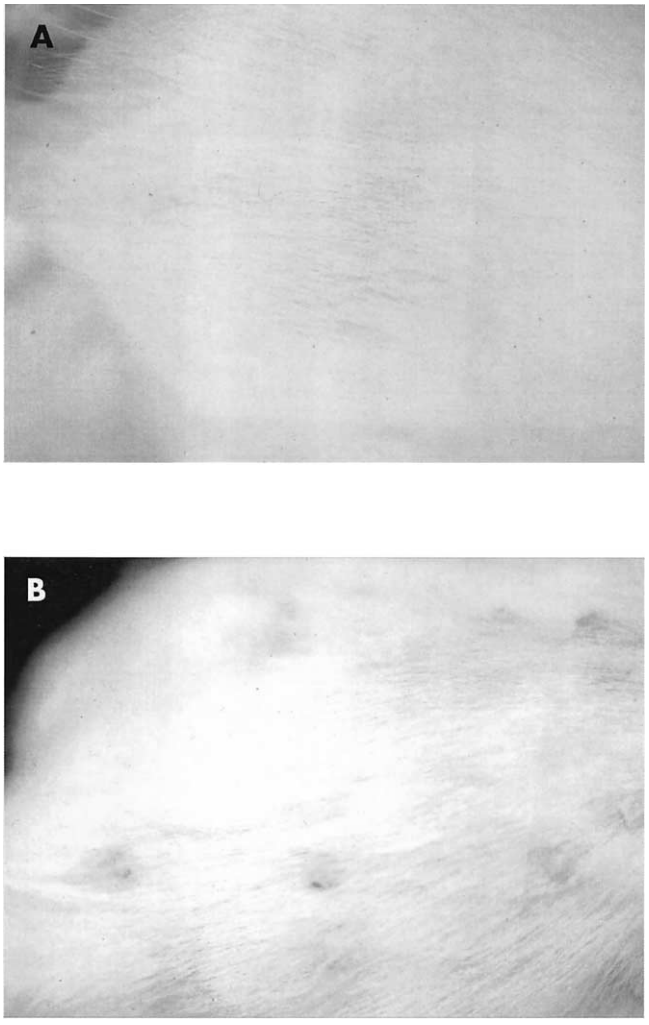

Fig. 1. Shaved dorsal region of a negative-control mouse (A) and a positive-control mouse (B).

3. Results and discussion

Figure 1 demonstrates the appearance of the shaved dorsal region of a negative-control mouse (top) and a positive-control mouse (bottom) after 18 weeks just before sacrifice. The distinctive nature of the tumors and the ease with which they can be counted is readily apparent. Animals were examined for tumors from the eighth week onwards at weekly intervals to assess progress, but accurate counts could not be made in the absence of skin shaving.

Figure 2 displays the percentage reduction in tumors related to the dose of each of the polyphenols used. Quercetin was clearly the most effective and gallic acid the least potent. No inhibition was seen up to a dose of 5 μmoles for the latter, whereas even at a dose of 1 μmole inhibition with the former was >90%. Intermediate efficacy was exhibited by (+)-catechin and *trans*-resveratrol. Probit analysis applied to these data utilizing Sigma Plot 5.0, SPSS, Inc., according to the statistical model of Lijinsky *et al.* [41] provided ED_{50} values of 5 and 6 μmoles, respectively for (+)-catechin and *trans*-resveratrol. For the remaining two polyphenols this could not be accurately calculated with the doses selected. To accomplish this, much lower doses of quercetin and somewhat higher doses of gallic acid will be required. However, on the basis of the results obtained using the present dose schedule, ED_{50} for quercetin is clearly well below 1 μmole, whereas for gallic acid it lies somewhere between 5 and 10 μmoles.

As another means of evaluating the response, the number of animals exhibiting one or more tumor at the end of the experiment was related to dosage for all four polyphenols (Table 1). Clearly, that number was much smaller for quercetin [4] than for (+)-catechin [12], gallic acid [12] or *trans*-resveratrol [15]. It should be emphasized that although the 5 μmole dose of gallic acid reduced by one the number of animals bearing tumors (Table 1), it did not reduce the average number of tumors per animal compared with the positive controls who had more tumors per animal when averaged out than the mice treated with 5 μmoles of gallic acid. The superiority of quercetin by this criterion was

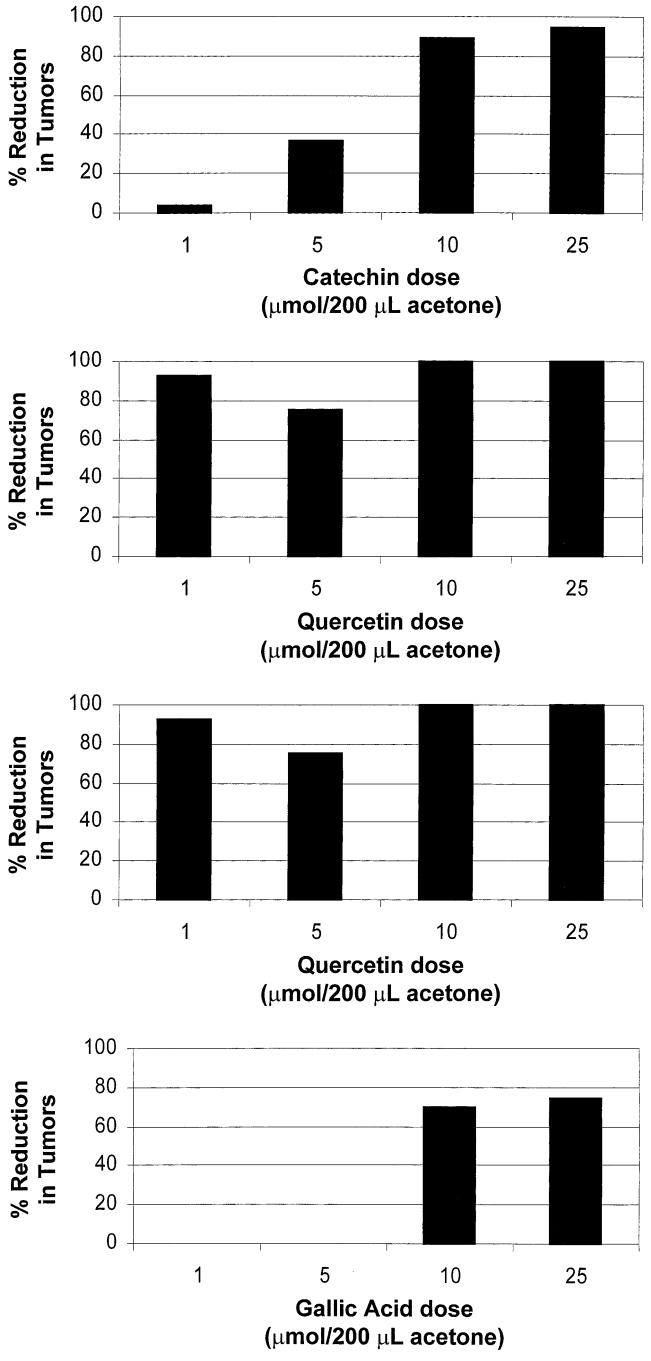

Fig. 2. Histograms showing relationship between percent reduction of mouse-skin tumors in relation to dose of the four polyphenols evaluated in this study.

statistically confirmed by the Fisher Exact Test ($p < 0.023$). While the present findings unequivocably point to quercetin as the most potent of the four compounds tested in this model, other considerations have to be taken into account in apportioning their relative contributions to the putative antitumorigenic potential of red wine.

First is their concentrations in red wine. These have been shown to vary widely depending upon cultivar, region and climatic conditions, as well as upon certain techniques used during the winemaking process [42–45]. As a rough generalization based upon data from laboratories (mostly our own) in which multiple polyphenols were simultaneously analyzed in large and representative surveys [46–50], the expected concentrations in a typical red wine would be in the order (+)-catechin (70 mg/L or 0.24 mmoles/L), gallic acid (25 mg/L or 0.16 mmoles/L), quercetin (6 mg/L or 0.018 mmoles/L) and resveratrol (6 mg/L or 0.026 mmoles/L). The last named value includes both isomers, since the *cis*- and *trans*-forms are in equilibrium and have similar activities in any biologic system in which both have been tested [51].

Table 1
No. of Mice Exhibiting One or More Tumors at 18 Weeks After Commencing Treatment With Various Concentrations of Polyphenols

Compound	Dose (μmoles/200 μL acetone)				
	0[a]	1	5	10	25
(+)-Catechin	4	4	5	2	1
Quercetin	4	1	3	0	0
trans-Resveratrol	5	5	4	3	3
Gallic Acid	5	5	4	3	0

[a] Positive controls

A second variable is the efficiency with which these polyphenols are absorbed from the human intestinal tract after wine consumption. We have recently reviewed evidence based upon both human and animal investigations, concluding that (+)-catechin and quercetin are poorly absorbed after oral doses [52]. We have also shown that >50% of tritiated-*trans*-resveratrol is absorbed after gavage in the rat [53]. Our latest report presents compelling evidence that, in man, *trans*-resveratrol is absorbed approximately 20-fold more effectively than (+)-catechin [54]. This absorption is dependent upon enzymatic sulfation and glucuronidation within the intestinal mucosa before entry into the portal blood [55–57]. Only a few percent of the circulating polyphenols and those excreted in human urine are in the nonconjugated form [54]. It is possible, but not yet proven, that this superior bioavailability will raise the blood concentrations of *trans*-resveratrol after wine consumption to the point where it attains a higher fraction of the ED_{50} for antitumorigenic activity than that reached by the other polyphenols with which it has been compared in this investigation. In offering this speculation, we recognize that we are extrapolating from the ED_{50} values calculated from the present data obtained with the mouse skin cancer model; no relevant experiments have been conducted on human cancer-bearing patients by us or other investigators. However, *in vitro* and *ex vivo* studies utilizing natural cancer cells or immortalized cancer cell-lines (see references cited in Introduction) have yielded comparable dose-response data for human and murine cancers. Nevertheless extrapolation of the present data, based on a small number of animals, to humans is not warranted in advance of more compelling confirmatory investigations involving larger numbers.

Acknowledgements

We thank the Canadian Vintners Association and the Natural Sciences and Engineering Research Council of Canada for a joint University-Industry Grant in support of this work that forms part of the thesis to be submitted by Mr. George Soleas in partial fulfillment of the requirements for the degree of Ph.D. at the University of Toronto. We are also grateful to Mrs. Sheila Acorn for her skillful preparation of this manuscript.

References

[1] Soleas GJ, Diamandis EP, Goldberg DM. Wine as a biological fluid: History, production, and role in disease prevention. J Clin Lab Anal 1997;11:287–313.
[2] Bradlow HL, Telang NT, Sepkovic DW, Osborne MP. Phytochemicals as modulators of cancer risk. Adv Exp Med Biol 1999;472:207–21.
[3] Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr 2001;21:381–406.
[4] Yoshida M, Sakai T, Hosokawa N, Marui N, Matsumoto K, Fujioka A, Nishino H, Aoike A. The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. FEBS Lett 1990; 260:10–13.
[5] Hosokawa N, Hosokawa Y, Sakai T, Yoshida M, Aoike A, Kawai K, Nishino H, Fukushima M. Inhibitory effect of quercetin on the synthesis of a possible cell-cycle-related 17 kDa protein in human colon cancer cells. Int J Cancer 1990;45:1119–24.
[6] Pawlikowska-Pawlega B, Jakubowicz-Gil J, Rzymowska J, Gawron A. The effect of quercetin on apoptosis and necrosis induction in human colon adenocarcinoma cell line LS180. Folia Histochem Cytobiol 2001;39:217–18.
[7] Kampa M, Hatzoglou A, Notas G, Damianaki A, Bakogeorgou E, Gemetzi C, Kouroumalis E, Martin PM, Castanas E. Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines. Nutr Cancer 2000;37:223–33.
[8] Damianaki A, Bakogeorgou E, Kampa M, Notas G, Hatzoglou A, Panagiotou S, Gemetzi C, Kouroumalis E, Martin PM, Castanas E. Potent inhibitory action of red wine polyphonols on human breast cancer cells. J Cell Biochem 2000;78:429–41.
[9] De S, Chakraborty J, Chakraborty RN, Das S. Chemopreventive activity of quercetin during carcinogenesis in cervix uteri in mice. Phytother Res 2000;14:347–51.
[10] Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, Aiello FB, Piantelli M. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. Int J Cancer 2000;87: 595–600.
[11] Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, Newmark HL, Bertagnolli MM. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. Carcinogenesis 2000;21:921–7.
[12] Ferry DR, Smith A, Malkhandi J, Fyfe DW, De Takats PG, Anderson D, Baker J, Kerr DJ. Phase I clinical trial of the flavonoid quercetin: Pharmacokinetics, and evidence for *in vivo* tyrosine kinase inhibition. Clin Cancer Res 1996;2:659–68.
[13] Mulholland PJ, Ferry DR, Anderson D, Hussain SA, Young AM, Cook JE, Hodgkin E, Seymour LW, Kerr DJ. Pre-clinical and clinical study of QC12, a water-soluble, pro-drug of quercetin. Ann Oncol 2001;12:245–8.
[14] Chung LY, Cheung TC, Kong SK, Fung KP, Choy YM, Chan ZY, Kwok TT. Induction of apoptosis by green tea catechins in human prostate cancer DU145 cells. Life Sci 2001;68:1207–14.
[15] Liu L, Castonguay A. Inhibition of the metabolism and genotoxicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rat hepatocytes by (+)-catechin. Carcinogenis 1991;12:1203–8.
[16] Weyant MJ, Carothers AM, Dannenberg AJ, Bertagnolli MM. (+)-Catechin inhibits intestinal tumor formation, and suppresses focal adhesion kinase activation in the min/+ mouse. Cancer Res 2001;61: 118–25.
[17] Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997;275:218–20.
[18] Uenobe F, Nakamura S-I, Miyazawa M. Antimutagenic effect of resveratrol against Trp-P-1. Mutat Res 1997;373:197–200.
[19] Hsieh TC, Burfeind P, Laud K, Backer JM, Traganos F, Darzynkiewicz Z, Wu JM. Cell cycle effects and control of gene expression by resveratrol in human breast carcinoma cell lines with different metastatic potentials. Int J Oncol 1999;15:245–52.
[20] Hiroyuki N, Yasuhiko K, Yoshiko U, Hideto S, Nobuaki S, Koshiro H, Airo T. Resveratrol inhibits human breast cancer cell growth and may mitigate the effect of linoleic acid, a potent breast cancer cell stimulator. J Cancer Res Clin Oncol 2001;127:258–64.
[21] Hsieh TC, Wu JM. Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. Exp Cell Res 1999;249:109–15.
[22] Ider Y, Vincent F, Duranton B, Badolo L, Gosse F, Bergmann C, Seiler N, Raoul F. Anti-proliferative effect of resveratrol, a natural component of grapes, and wine, on human colonic cancer cells. Cancer Lett 2000;158:85–91.
[23] Mutoh M, Takahashi M, Fukuda K, Matsushima-Hibiya Y, Mutoh H, Sugimura T, Wakabayashi K. Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. Carcinogenesis 2000;21:959–63.
[24] Elattar TM, Virji AS. The effect of red wine and its components on growth and proliferation of human oral squamous carcinoma cells. Anticancer Res 1999;19:5407–14.
[25] Surh YJ, Hurh YJ, Kang JY, Lee E, Kong G, Lee SJ. Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyelocytic leukemia (HL-60) cells. Cancer Lett. 1999;140:1–10.
[26] Huang C, Ma WY, Goranson A, Dong Z. Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. Carcinogenesis 1999;20:237–42.
[27] Tsan MF, White JE, Maheshwari JG, Bremner TA, Sacco J. Resveratrol induces Fas signalling-independent apoptosis in THP-1 human monocytic leukaemia cells. Br J Haematol 2000;109:405–12.
[28] Ahmad N, Adhami VM, Afaq F, Feyes DK, Mukhtar H. Resveratrol causes WAF-1/p21-mediated G_1-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. Clin Cancer Res 2001;7:1466–73.
[29] Ciolino HP, Daschner PJ, Yeh GC. Resveratrol inhibits transcription of CYP1A1 in vitro by preventing activation of the aryl hydrocarbon receptor. Cancer Res 1998;58:5707–12.
[30] Casper RF, Quesne M, Rogers IM, Shirota T, Jolivet A, Milgrom E, Savouret JF. Resveratrol has antagonist activity on the aryl hydrocarbon receptor: Implications for prevention of dioxin toxicity. Mol Pharmacol 1999;56:84–90.
[31] Kimura Y, Okuda H. Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth, and metastasis to lung, and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. J Nutr 2001;131:1844–9.
[32] Serrano A, Palacios C, Roy G, Cespon C, Villar ML, Nocito M, Gonzalez-Porque P. Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys 1998;350:49–54.

[33] Roy G, Lombardia M, Palacios C, Serrano A, Cespon C, Ortega E, Eiras P, Lujan S, Revilla Y, Gonzalez-Porque P. Mechanistic aspects of the induction of apoptosis by lauryl gallate in the murine B-cell lymphoma line Wehi 231. Arch Biochem Biophys 2000;383:206–14.
[34] Ohno Y, Fukuda K, Takemura G, Toyota M, Watanabe M, Yasuda N, Xinbin Q, Maruyama R, Akao S, Gotou K, Fujiwara T, Fujiwara H. Induction of apoptosis by gallic acid in lung cancer cells. Anticancer Drugs 1999;10:845–51.
[35] Yoshioka K, Kataoka T, Hayashi T, Hasegawa M, Ishi Y, Hibasami H. Induction of apoptosis by gallic acid in human stomach cancer KATO 111 and colon adenocarcinoma COLO 205 cell lines. Oncol Rep 2000:7;1221–3.
[36] Saeki K, Yuo A, Isemura M, Abe II, Seki T, Noguchi H. Apoptosis-inducing activity of lipid derivatives of gallic acid. Biol Pharm Bull 2000;23:1391–4.
[37] Liang YC, Lin-Shiau SY, Chen CF, Lin JK. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (-)-epigallocatechin-3-gallate. J Cell Biochem 1999; 75:1–12.
[38] Muto S, Yokoi T, Gondo Y, Katsuki M, Shioyama Y, Fujita K, Kamataki T. Inhibition of benzo[a]pyrene-induced mutagenesis by (-)-epigallocatechin gallate in the lung of rpsL transgenic mice. Carcinogenesis 1999;20:421–4.
[39] Li HC, Yashiki S, Sonoda J, Lou H, Ghosh SK, Byrnes JJ, Lema C, Fujiyoshi T, Karasuyama M, Sonoda S. Green tea polyphenols induce apopotosis *in vitro* in peripheral blood T lymphocytes of adult T-cell leukemia patients. Jpn J Cancer Res 2000;91:34–40.
[40] Gupta S, Ahmad N, Nieminen AL, Mukhtar H. Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (-)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol Appl Pharmacol 2000;164:82–90.
[41] Lijinsky W, Kavatch RM, Riggs CW, Walters PT. Dose-response study with N-nitrosomorpholine in drinking water of F-344 rats. Cancer Res 1988;48:2089–95.
[42] Mattivi F. Nicolini G. Influenza della tecnica di vinificasione sul contenuto di resveratrolo dei vini. L'Enotecnico 1993;July-August: 81–88.
[43] Jeandet P, Bessis R, Sbaghi M, Meunier P, Trollat P. Resveratrol content of wines of different ages: relationship with fungal disease pressure in the vineyard. Am J Enol Vitic 1995;46:1–4.
[44] Jeandet P, Bessis R, Maume BF, Meunier P, Peyron D, Trollat P. Effect of enological practices on the resveratrol isomer content of wine. J Agric Food Chem 1995;43:316–19.
[45] Soleas GJ, Goldberg DM, Karumanchiri A, Diamandis EP, Ng E. Influences of viticultural, and oenological factors on changes in *cis*- and *trans*-resveratrol in commercial wines. J Wine Res 1995;6:107–21.
[46] Goldberg DM, Yan J, Ng E, Diamandis EP, Karumanchiri A, Soleas GJ, Waterhouse AL. A global survey of *trans*-resveratrol concentrations in commercial wines. Am J Enol Vitic 1995;46:159–65.
[47] Goldberg DM, Ng E, Yan J, Karumanchiri A, Soleas GJ, Diamandis EP. Regional differences in resveratrol isomer concentrations of wines from various cultivars. J Wine Res 1996;7:13–24.
[48] Soleas GJ, Dam J, Carey M, Diamandis EP, Goldberg DM. Towards the chemical fingerprinting of wines; Cultivar-related patterns of polyphenolic constituents in Ontario wines. J Agric Food Chem 1997;45:3871–80.
[49] Goldberg DM, Karumanchiri A, Tsang E, Diamandis EP, Soleas GJ. Catechin and epicatechin concentrations of red wine. Regional and cultivar-related differences. Am J Enol Vitic 1997;49:23–34.
[50] Goldberg DM, Tsang E, Karumanchiri A, Soleas GJ. Quercetin and p-coumaric acid concentrations in commercial wines. Am J Enol Vit 1998;49:142–51.
[51] Soleas GJ, Diamandis EP, Goldberg DM. The world of resveratrol. Adv Exp Biol Med 2001;492:159–82.
[52] Soleas GJ, Yan J, Goldberg DM. Measurement of *trans*-resveratrol, (+)-catechin and quercetin in rat and human blood and urine by gas chromatography with mass selective detection. Methods Enzymol 2001;335:130–45.
[53] Soleas GJ, Angelini M, Grass L, Diamandis EP, Goldberg DM. Absorption of *trans*-resveratrol in rats. Methods Enzymol 2001;335: 145–54.
[54] Soleas GJ, Yan J, Goldberg DM. Ultrasensitive assay for three polyphenols (catechin, quercetin, and resveratrol), and their conjugates in biological fluids utilizing gas chromatography with mass selective detection. J Chromatogr B 2001;757:161–72.
[55] Pagana G, Rice-Evans CA. The identification of flavonoids as glycosides in human plasma. FEBS Let 1997;401:78–82.
[56] Morand C, Crespy V, Manach C, Besson C, Demigne C, Remesy C. Plasma metabolites of quercetin, and their antioxidant properties. Am J Physiol 1998;275:R212–19.
[57] Crespy V, Morand C, Manach C, Besson C, Demigne C, Remesy C. Part of quercetin absorbed in the small intestine is conjugated, and further secreted in the intestinal lumen. Am J Physiol 1999;277: G120–6.