Anti-Cancer Drugs 1999, **10**, pp. 187–193

Research paper

Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation

Tawfik MA ElAttar and Adi S Virji
Hormone Research Laboratory, University of Missouri–Kansas City, Schools of Dentistry and Medicine, Kansas City, MI 64108, USA.

Resveratrol and quercetin are polyphenols which have been detected in significant amounts in green vegetables, citrus fruits and red grape wines. Beneficial effects attributed to these compounds include anti-inflammatory, antiviral and antitumor properties. The effect of resveratrol and quercetin on growth of human oral cancer cells is unknown. Resveratrol and quercetin, in concentrations of 1 to 100 μM, were incubated in triplicates with human oral squamous carcinoma cells SCC-25 in DMEM-HAM's F-12 supplemented with fetal calf serum and antibiotics in an atmosphere of 5% CO_2 in air at 37°C for 72 h. Cell growth was determined by counting the number of viable cells with a hemocytometer. Cell proliferation was measured by means of incorporation of [^{3}H]thymidine in nuclear DNA. Resveratrol at 10 and 100 μM induced significant dose-dependent inhibition in cell growth as well as in DNA synthesis. Quercetin exhibited a biphasic effect, stimulation at 1 and 10 μM, and minimal inhibition at 100 μM in cell growth and DNA synthesis. Combining 50 μM of resveratrol with 10, 25 and 50 μM of quercetin resulted in a gradual and significant increase in the inhibitory effect of quercetin on cell growth and DNA synthesis. We conclude that resveratrol or a combination of resveratrol and quercetin, in concentrations equivalent to that present in red wines, are effective inhibitors of oral squamous carcinoma cell (SCC-25) growth and proliferation, and warrant further investigation as cancer chemopreventive agents. [© 1999 Lippincott Williams & Wilkins.]

Key words: Quercetin, resveratrol, squamous cell carcinoma.

Supported by the Rinehart Foundation Grant K-2-10573-5143 for cancer research.

Correspondence to TMA ElAttar, Hormone Research Laboratory, University of Missouri–Kansas City, 650 E 25th Street, Kansas City, MI 64108-2784, USA.
Tel: (+1) 816 235-2073; Fax: (+1) 816 235-2157;
E-mail: virjia@umkc.edu

Introduction

Flavonoids are naturally occurring diphenylpropanoids which occur primarily as glycosides in plant foods. They appear in animal and human cells following consumption of vegetables, fruits, and beverages such as tea and wine.[1,2] Interest in flavonoids has increased because of their potential role in prevention of human cancer.[1]

Flavonoids are classified into two major groups: the anthocyanins and anthroxanthines. The anthroxanthines are subdivided into the flavones, flavonones and flavonols.[2] Flavonols, such as quercetin, myricetin, isorhamnetin and kaemferol, and the corresponding flavonones, apigenin and luteolin, have antioxidant properties,[3] and their consumption has been associated with a reduced risk of cancer,[4,5] thrombosis[6,7] and cardiovascular disease.[8,9]

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a common dietary component. It has been stated that quercetin and other flavonoids present in foods cannot be absorbed in the intestines because they are bound to sugars as glycosides and that there are no intestinal enzymes that can split these glycosidic bonds. Only free flavonoids can be absorbed.[10,11]

In several *in vitro* experiments quercetin showed growth inhibitory effects on various human cancer cell lines such as colon,[12] breast,[13,14] ovarian,[15] gastrointestinal[16] and leukemic.[17] Recently quercetin has been shown to down-regulate signal transduction in human breast carcinoma cells.[18]

Resveratrol (3,4′,5-trihydroxystilbene) is a natural phytoalexin non-flavonoid phenol which was identified in the skin of red grapes and grape products.[19] Its physiological function is thought to reside in the protection of plants from environmental stress and fungal infection. Resveratrol has been suggested to play a role in the prevention of heart disease associated with

red wine consumption, as it inhibits the synthesis of thromboxane in platelets,[20] leukotrienes in neutrophils[21] and biological oxidation reactions associated with the generation of free radicals.[22] Resveratrol was also found to be antimutagenic,[23] and can inhibit cellular events associated with tumor initiation, promotion and progression.[24]

Resveratrol and quercetin are major components of red wines. The average concentration of resveratrol in commonly available red wines is approximately 2.0-40.0 μM, while that of quercetin is approximately 0.4-40 μM.[25]

Although the various biological activities of flavonoids, including anti-inflammatory, cytostatic, cytotoxic, anticancer and antiviral properties, have been shown only with individual flavonoids, the effect of combinations of quercetin and resveratrol has not yet been established. Therefore, the goal of this research was to (i) investigate the effect of each of resveratrol and quercetin, alone and in combination on the growth and proliferation of human oral squamous carcinoma cell line SCC-25; and (ii) compare the efficacy of resveratrol and quercetin with that of classical chemotherapeutic agents, indomethacin and cisplatin, in inhibition of growth and proliferation of cancer cells.

Materials and methods

Human tongue squamous carcinoma cells (SCC-25) were purchased from ATCC (Rockville, MD). The cell line was developed by Rheinwald and Beckett.[26] Cisplatin, indomethacin, resveratrol and quercetin were obtained from Sigma (St Louis, MO). Cells were grown in DMEM-HAM's Nutrient F-12 (50:50) supplemented with 10% fetal calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml) and hydrocortisone (0.4 mg/ml). The medium was changed 3 times weekly; subcultures were initiated by transfer of about 2×10^6 cells to culture flasks and then cultured at 37°C in a water-saturated atmosphere of 5% CO_2 in air.

Measurement of cell growth

Cells were used during the exponential growth curve. Cells were cultured in triplicates starting at an initial density of 260×10^3/ml medium for 72 h in the presence of test agents. At the end of the incubation period, adherent cells were trypsinized and viable cells were counted in a hemocytometer.

Measurement of cell proliferation (DNA synthesis)

Cell proliferation inhibition is almost invariably associated with a decrease in DNA synthesis. Determination of DNA synthesis was performed as previously described.[27]

Cell viability

Cell viability was determined by incubating treated cells in 0.1% (w/v) Trypan blue in PBS, and counting the total number of cells and the number of those excluding the stain (the viable ones), using a hemocytometer. If cell viability in treated cultures was lower than 80%, then it is not valid to refer to the effect of test agents as growth inhibition but rather a cytotoxic effect.

Statistical analysis

The results are presented as means $\pm$ SEM of three experiments, each performed in triplicate. Student's *t*-test was used to evaluate the statistical significance of the difference between experimental and control groups.

Results

Treatment of oral squamous carcinoma cells (SCC-25) with indomethacin, cisplatin, resveratrol, quercetin, and a combination of resveratrol and quercetin produced the following effects on cell growth and DNA synthesis.

Indomethacin

At 0.1-10 μM indomethacin induced significant dose-dependent inhibition in cell growth (35.0 ± 6.7-50.0 ± 4.4%) as well as in DNA synthesis (0.0 ± 0.9-45.0 ± 7.5%) (Table 1, Figures 1 and 2).

Cisplatin

At 0.1-10 μM cisplatin induced significant dose-dependent inhibition in cell growth which varied from 68.0 ± 1.0 to 97.0 ± 0.5%. At same concentration range cisplatin caused significant dose-dependent inhibition in DNA synthesis (0.6 ± 7.4-76.0 ± 0.7%) (Table 1, Figures 1 and 2).

Table 1. Effect of indomethacin, cisplatin, resveratrol, quercetin and resveratrol–quercetin combinations on cell growth (cell count) and incorporation of [^{3}H]thymidine (DNA synthesis) in SCC-25 per culture flask

Concentration (μM)	Cell growth				DNA			
	% Inhibition	% Stimulation	±SEM	p	% Inhibition	% Stimulation	±SEM	p
Indomethacin								
0.1	35.0	–	6.7	<0.02	0	–	0.9	>0.2
1.0	42.0	–	5.9	<0.01	26.0	–	1.1	<0.01
10.0	50.0	–	4.4	<0.01	45.0	–	7.5	<0.02
Cisplatin								
0.1	68.0	–	1.0	<0.001	0.6	–	7.4	>0.2
1.0	89.0	–	0.6	<0.001	21.0	–	3.4	<0.2
10.0	97.0	–	0.5	<0.001	76.0	–	0.7	<0.001
Resveratrol								
1.0	–	1.9	1.2	>0.2	–	2.0	4.0	>0.2
10.0	13.6	–	1.2	<0.02	13.0	–	2.4	<0.02
100.0	99.5	–	0.1	<0.001	99.0	–	0.4	<0.001
Quercetin								
1.0	–	36.2	3.0	<0.01	–	24.0	2.4	<0.1
10.0	–	40.8	2.7	<0.001	–	11.0	4.6	<0.2
100.0	12.3	–	2.7	<0.2	2.3	–	1.5	>0.2
Resveratrol (R) + Quercetin (Q)								
R(50) + Q(10)	45.3	–	2.1	<0.01	–	24.0	4.6	<0.1
R(50) + Q(25)	47.7	–	2.0	<0.01	25.0	–	2.0	<0.02
R(50) + Q(50)	62.0	–	0.7	<0.01	56.0	–	1.6	<0.001

Values represent the mean ± SEM of percent inhibition or percent stimulation of three replicate cultures within each experiment. p values are compared to corresponding control groups. Dashes represent no effect.

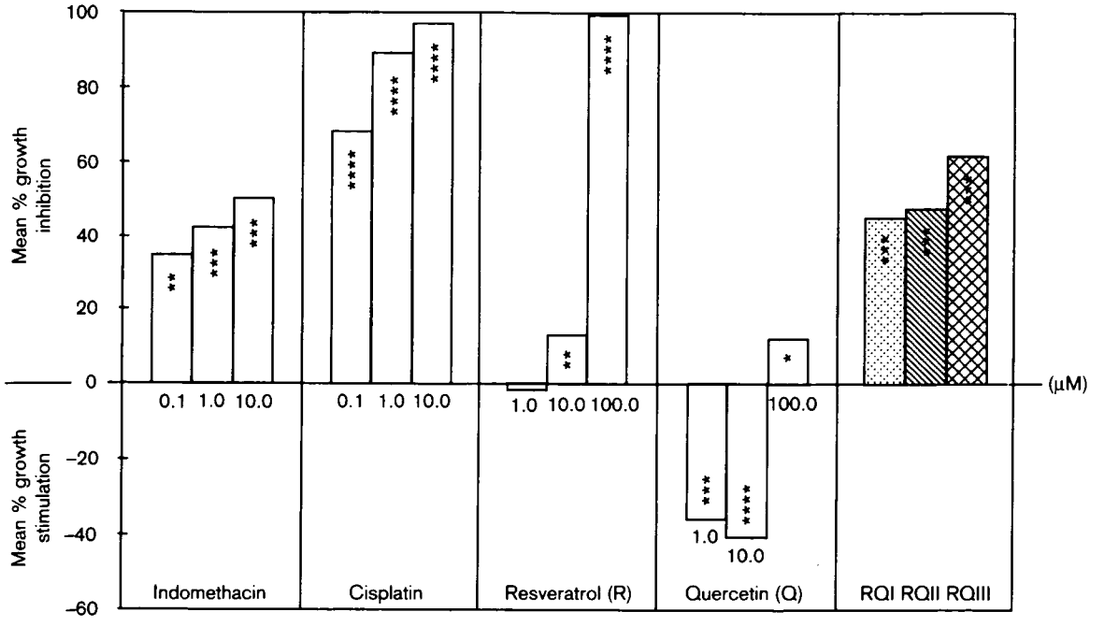

Figure 1. Effect of indomethacin, cisplatin, resveratrol, quercetin and resveratrol–quercetin combinations on cell growth (cell count) of human oral squamous carcinoma cell, SCC-25. RQI R (50 μM) + Q (10 μM); RQII R (50 μM) + Q (25 μM); RQIII R (50 μM) + Q (50 μM). *p<0.2; **p<0.1; ***p<0.02; ****p<0.01.

Resveratrol

At 10 and 100 μM resveratrol induced significant inhibition in cell growth (13.6$\pm$1.2 and 99.5$\pm$0.1%) and DNA synthesis (13.0$\pm$2.4 and 99.0$\pm$0.4%) (Table 1, Figures 1 and 2).

Quercetin

At 1 and 10 μM quercetin caused significant stimulation (36.2$\pm$3.0 and 40.8$\pm$2.7%) in cell growth as well as DNA synthesis (24.0$\pm$2.4 and 11.0$\pm$4.6%). However, at 100 μM quercetin caused significant inhibition (12.3$\pm$2.7%) in cell growth but not in DNA synthesis (2.3$\pm$1.5%) (Table 1, Figures 1 and 2).

Combinations of resveratrol and quercetin

Resveratrol at 50 μM when combined with 10, 25 or 50 μM of quercetin caused significant inhibition in cell growth which varied between 45.3$\pm$2.1 and 62.0$\pm$0.7%. In DNA studies 50 μM of resveratrol combined with 10 μM of quercetin induced significant stimulation (24.0$\pm$4.6%). However, when 25 or 50 μM of quercetin were combined with 50 μM of resveratrol, it caused significant inhibition in DNA synthesis (25.0$\pm$2.0 and 56.0$\pm$1.6, respectively (Table 1, Figures 1 and 2).

Discussion

The data reported in the present study have shown that resveratrol at the concentration of 1 μM had negligible stimulatory effect, at 10 μM had some demonstrable inhibitory effect, and at 100 μM had significant inhibitory effect on both cell growth and DNA synthesis. On the other hand, quercetin at 1 and 10 μM concentrations induced significant stimulatory effect, while at 100 μM it caused minimal inhibitory effect on cell growth as well as DNA synthesis (Table 1).

Since resveratrol and quercetin exist in combination with other flavonoids in fruits and vegetables, we studied the effect of three concentrations of resveratrol and quercetin similar to that found in red wines.[25] When quercetin in 10, 25 and 50 μM concentrations was added to 50 μM of resveratrol, gradual and significant inhibition in cell growth took place (Table 1). However, in the DNA study the combination of quercetin and resveratrol had a biphasic effect: stimulatory when the ratio of quercetin to resveratrol

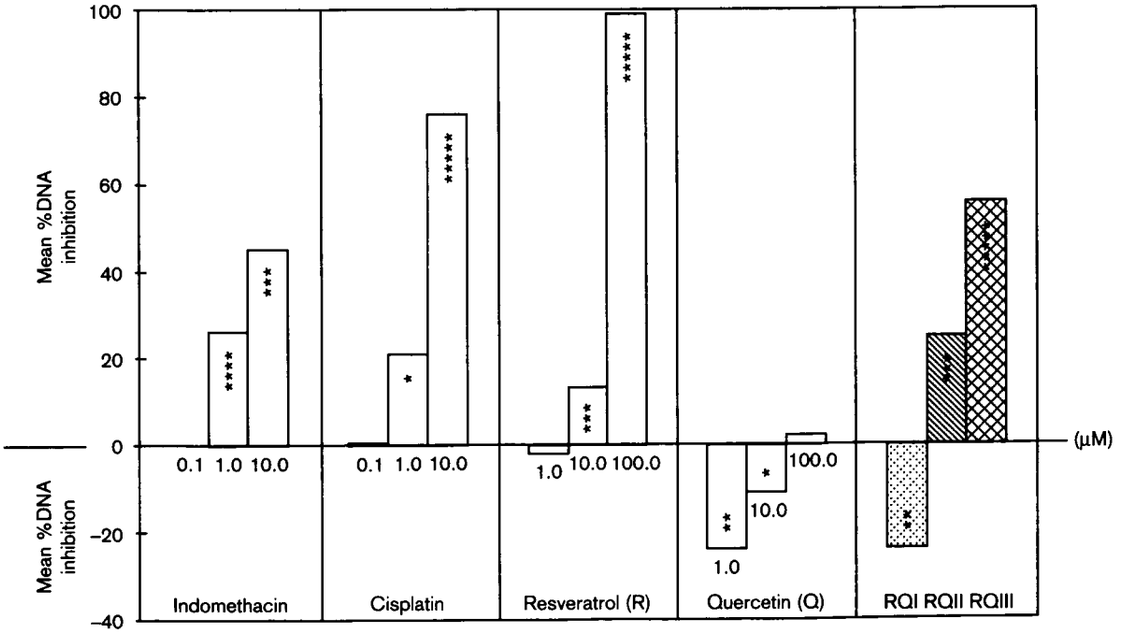

Figure 2. Effect of indomethacin, cisplatin, resveratrol, quercetin and resveratrol–quercetin combinations on incorporation of [^{3}H]thymidine (DNA synthesis) in human oral squamous carcinoma cell, SCC-25. RQI ▨ R (50 μM) + Q (10 μM); RQII ▧ R (50 μM) + Q (25 μM); RQIII ▩ R (50 μM) + Q (50 μM). *$p<0.2$; **$p<0.1$; ***$p<0.02$; ****$p<0.01$; *****$p<0.001$.

was 1:5, and inhibitory when the ratios were 1:2 and 1:1 (Table 1).

Indomethacin and cisplatin in a concentration range of 0.1–10 μM induced significant dose-dependent inhibition in cancer cell growth as well as DNA synthesis. Cisplatin at 10 μM concentration was twice as potent as indomethacin and 10-fold as potent as resveratrol in inhibition of cancer cell growth. Resveratrol at 100 μM concentration had the same effect as that of cisplatin and 2-fold the effect of indomethacin, both at 10 μM, on inhibition of cancer cell growth.

The only study in the literature on the effect of quercetin on head and neck cancer was that of Castillo.[28] It was found that quercetin at concentrations of 110 μM and above caused significant inhibition in squamous cell carcinoma of the tongue. In the same paper the investigators discovered that quercetin failed to inhibit lung metastasis induced by $B_{16}F_{10}$ melanoma cells. There was no relationship between antioxidant activity of various polyphenolic compounds and metastasis.[28]

The biological mechanisms by which flavonoids may delay or inhibit onset of certain tumors are currently unknown. However, they may act as *in vivo* antioxidants by inhibiting mutagenicity and damage to DNA, by altering eicosanoid synthesis, and by preventing or halting the spread of nascent tumors via alterations in platelet aggregation and angiogenesis.[29–33]

The flavonoids quercetin, luteolin and genistein were found to scavenge oxygen free radicals (H_2O_2, O_2), inhibit lipid peroxidation, and quench the formation of 8-hydroxy-2′-deoxyguanosine by UV light irradiation and fenton reaction.[34] The scavenging of oxygen free radicals and subsequent protection of cellular macromolecules against oxidative damage may be responsible for the anticarcinogenic and chemopreventive effects of these flavonoids.

Quercetin has been shown to have growth inhibitory effect on human colon cancer cells via the inhibition of cell-cycle-related 17 kDa protein which blocks cell transition from G_0/G_1 into the S phase of the cycle.[35] Quercetin has also been reported to induce apoptosis in several cell lines of human tumor cells via inhibition of heat shock proteins.[36,37] There are also several biological mechanisms by which quercetin could inhibit growth of cancer cells such as inhibition of macromolecules synthesis,[38] glycolysis,[39] lactate transport[40] and several protein kinases.[41]

Resveratrol inhibited, in a dose-dependent manner, free radical formation when human promyelocytic leukemia (HL-60) cells were treated with 12-*O*-tetrahydrodecanoylphorbol-13-acetate (TPA).[42]The compound also functioned as an antimutagen, as illustrated by its dose-dependent inhibition of mutagenic response induced by treatment of *Salmonella typhinurium* strain TM677 with a 7,12-dimethylbenz[*a*]-anthracene (DMBA).[23] This illustrates the potential cancer chemopreventive activity of resveratrol. In addition, resveratrol induced quinone reductase activity with cultured mouse hepatoma (Hepa 1c1c7) cells, which is relevant because phase II enzymes, such as quinone reductase, are capable of metabolically detoxifying carcinogens.[43]

Inhibitors of ribonucleotide reductase, the enzyme that catalyzes the reductive conversion of ribonucleotides to deoxyribonucleotides, have been shown to be potential anticancer, antibacterial and antiviral agents.[45–47] Resveratrol was found to be more potent inhibitor of ribonucleotide reductase in mammalian cells than hydroxyurea and 4-hydroxyanisole, the only ribonucleotide reductase tyrosyl radical scavengers used clinically as anticancer agents.[47] Tyrosyl radical scavenging results in inactivation of ribonucleotide reductase and inhibition of DNA synthesis. The antiproliferative properties of resveratrol are likely to be due to its ability to efficiently scavenge the essential tyrosyl radical and consequently to inhibit DNA synthesis.[47]

In conclusion the data suggest that resveratrol or a combination of resveratrol and quercetin, in concentrations equivalent to that present in red wines, are effective inhibitors of oral cancer cell growth and proliferation (DNA synthesis). In nature, resveratrol and quercetin have been known to exist in various ratios. It becomes imperative then to study the effect of combinations of these two compounds as they coexist in nature. Our data strongly suggests that the study of such combinations may prove to be effective in chemoprevention or treatment of cancer.

References

1. Hertog MGL, Hollman PCH, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutr Cancer* 1993; **20**: 21–9.
2. Das DK. Naturally occurring flavonoids: structure, chemistry, and high performance liquid chromatography methods for separation and characterization. *Methods Enzymol* 1994; **234**: 410–20.
3. Shahidi F, Wanasundara PK. Phenolic antioxidants. *Crit Rev Food Sci Nutr* 1992; **32**: 67–103.
4. Wattenberg LW. Inhibition of carcinogenesis by minor anutrient constituents of the diet. *Proc Nutr Soc* 1990; **49**: 173–83.

5. Wei H, Tye L, Bresnick E, Birt DF. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. *Cancer Res* 1990; **50**: 499-502.
6. Gryglewski RJ, Korbut R, Robak J, Sweis J. On the mechanism of antithrombotic action of flavonoids. *Biochem Pharmacol* 1987; **36**: 317-22.
7. Laughton MJ, Evans PJ, Moroney MA, Hoult JRS, Halliwell B. Inhibition of mammalian 5-lipooxygenase activity and cyclooxygenase by flavonoids and phenolic dietary additives—relationship to antioxidant activity and iron ion-reducing ability. *Biochem Pharmacol* 1991; **42**: 1673-81.
8. Hertog MGL, Hollman PCH. Potential health effects of the dietary flavonol quercetin. *Eur J Clin Nutr* 1996; **50**: 63-71.
9. Frankel EN. Potential health benefits of grape and wine antioxidants. In: Stockley CS, eds. *Medically, is wine just another alcoholic beverage (Proc Wolf Blass Foundation Int Wine and Health Conf)* 1996: 45-9.
10. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 1976; **24**: 117-91.
11. Harborne JB. In: Cody V, Middleton E, Harborne JB, eds. *Plant flavonoids in biology and medicine: Biochemical, pharmacological and structure-activity relationships*. New York: Alan R Liss 1986: 15.
12. Ranelletti FO, Ricci R, Larocca LM, *et al.* Growth-inhibitory effect of quercetin and presence of type-II estrogen-binding sites in human colon cancer cell lines and primary colorectal tumors. *Int J Cancer* 1992; **50**: 486-92.
13. Scambia G, Ranelletti FO, Benedetti Panici P, *et al.* Quercetin inhibits the growth of a multidrug-resistant estrogen-receptor-negative MCF-7 human breast cancer cel line expressing type II estrogen-binding sites. *Cancer Chemother Pharmacol* 1991; **28**: 255-8.
14. Albert Yeh Y, Herenyiova M, Weber G. Quercetin: synergistic action with carboxyamidotriazole in human breast carcinoma cells. *Life Sci* 1995; **57**: 1285-92.
15. Scambia G, Ranelletti FO, Benedetti Panici P, *et al.* Inhibitory effect of quercetin on OVCA 433 cells and presence of type II estrogen binding sitesin primary ovarian tumors and cultured cells. *Br J Cancer* 1990; **62**: 942-7.
16. Yoshida M, Sakai T, Hosokawa N, Marui N, *et al.* The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. *FEBS Lett* 1990; **260**: 10-3.
17. Yoshida M, Yamamoto M, Nikaido T. Quercetin arrests human leukemic T-cells in late G_1 phase of the cell cycle. *Cancer Res* 1992; **52**: 6676-81.
18. Singhal RL, Albert Yeh Y, Prajda N, Olah E, Sledge GW Jr, Weber G. Quercetin down-regulates signal transduction in human breast carcinoma cells. *Biochem Biophys Res Commun* 1995; **208**: 425-31.
19. Greasy LL, Coffee M. Phytoalexin production potential of grape berries. *J Am Soc Hortic Sci* 1988; **113**: 230-4.
20. Pace-Asiak CR, Hahn S, Diamandis E, Soleas G, Goldberg DM. The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin Chim Acta* 1995; **235**: 207-19.
21. Goldberg DM, Soleas GJ, Hahn SE, Diamandis EP, Karumanchiri A. Identification and assay of trihydroxystilbenes in wine and their biological properties. In: Wakins T, ed. *Wine nutritional and therapeutic benefits*. Washington, DC: ACS 1996: 24-43.
22. Frankel EN, Waterhouse AL, Kinsella JE. Inhibition of human LDL oxidation by resveratrol. *Lancet* 1993; **341**: 1103-4.
23. Shamon LA, Chen C, Mehta RG, Steele V, Moon RC, Pezzuto JM. A correlative approach for the identification of antimutagens that demonstrate chemopreventive activity. *Anticancer Res* 1994; **14**: 1775-8.
24. Jang M, Cai L, Udeani GO, *et al.* Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997; **275**: 218-20.
25. McMurtrey KD. Resveratrol in wine. In: Watkins T, ed. *Wine nutritional and therapeutic benefits*. Washington, DC: ACS 1996: 44-55.
26. Rheinwald JG, Beckett MA. Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultures from human squamous cell carcinomas. *Cancer Res* 1981; **41**: 1657-63.
27. ElAttar TMA, Lin HS. Vitamin E succinate potentiates the inhibitory effect of prostaglandins on oral squamous carcinoma cell proliferation. *Prostaglandins Leukotrienes and Essential Fatty Acids* 1995; **52**: 69-73.
28. Castillo MH, Perkins E, Campbel JH, *et al.* The effect of bioflavonoid quercetin on squamous cell carcinoma of head and neck origin. *Am J Surg* 1989; **158**: 351-5.
29. Osawa T. Phenolic antioxidants in dietary plants as antimutagens. In: Huang HT, Ho CT, Lee CY, eds. *Phenolic compounds in food and their effects on health*. Washington, DC: ACS 1992; **2**: 135-49.
30. Ito N, Hirose M, Shirai T. Carcinogenicity and modification of carcinogenic response by plant phenols. In: Huang HT, Ho CT, Lee CY, eds. *Phenolic compounds in food and their effects on health*. Washington, DC: ACS 1992; **2**: 269-83.
31. Yoshizawa S. Horiuchi T, Suganuma M, *et al.* Penta-*O*-galloyl-b-D-glucose and (−)-epigallocatechin gallate: cancer preventive agents. In: Huang HT, Ho CT, Lee CY, eds. *Phenolic compounds in food and their effects on health*. Washington, DC: ACS 1992; **2**: 316-25.
32. Kelloff GJ, Boone CW, Steele VE, *et al.* Mechanistic considerations in chemopreventive drug development. *J Cell Biochem Suppl* 1994; **20**: 1-24.
33. Stavric B. Quercetin in our diet: from potent mutagen to possible anticarcinogen. *Clin Biochem* 1994; **27**: 245-8.
34. Cai Q, Rahn KO, Zhang R. Dietary flavonoids, quercetin, luteolin and genistein, reduce oxidative DNA damage and lipid peroxidation and quench free radicals. *Cancer Lett* 1997; **119**: 99-107.
35. Hosokawa N, Hosokawa Y, Sakai T, Yoshida M. Inhibitory effect of quercetin on the synthesis of a possibly cell-cycle -related 17-kDa protein in human colon cancer cells. *Int J Cancer* 1990; **45**: 1119-24.
36. Wei YQ, Zhao X, Kariya Y, Fukata H, Teshigawara K, Uchida A. Induction of apoptosis by quercetin: involvement of heart shock protein. *Cancer Res* 1994; **54**: 4952-7.

37. Elia G, Santoro MG. Regulation of heat shock protein synthesis by quercetin in human erythroleukemia cells. *Biochem J* 1994; **300**: 201-9.
38. Giaziani Y, Chayoth R. Regulation of cyclic AMP level and synthesis of DNA, RNA and protein by quercetin in Ehrlich ascites tumor cells. *Biochem Pharmacol* 1979; **28**: 397-403.
39. Suolinna EM, Buchsbaum RN, Racker E. The effect of flavonoids on aerobic glycolysis and growth of tumor cells. *Cancer Res* 1975; **35**: 1865-72.
40. Kim JH, Kim SH, Alfieri AA, Young CW. Quercetin, an inhibitor of lactase transport and a hyperthermic sensitizer of Hela cells. *Cancer Res* 1984; **44**: 102-6.
41. End DW, Look RA, Shafer NL, Balles EA, Persico FJ. Nonselective inhibition of mammalian protein kinases by flavonoids *in vitro*. *Res Commun Pathol Pharmacol* 1987; **56**: 75-86.
42. Sharma S, Stutzman JD, Kelloff GJ, Steele VE. Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. *Cancer Res* 1994; **54**: 5848-55.
43. Prochaska HJ, Santamaria AB. Direct measure of NADPH quinone reductase from cells cultured in microtiter wells. A screening assay for anticarcinogenic enzyme inducers. *Anal Biochem* 1988; **169**: 328-36.
44. Gao WY, Cara A, Gallo RC, Lori F. Low levels of deoxynucleotides in peripheral blood lymphocytes: a strategy to inhibit human immunodeficiency virus-type I replication. *Proc Natl Acad Sci USA* 1993; **90**: 8925-8.
45. Lori F, Malykh A, Cara A, Sun D. Weinstein JN, Lisziewicz J, Gallo RC. Hydroxyurea as an inhibitor of human immunodeficiency virustype 1 replication. *Science* 1994; **266**: 801-5.
46. Gao WY, Johns DG, Chokekijchai S, Mitsuya H. Disparate actions of hydroxyurea in potentiation of purine and pyrimidine 2^1, 3^1-dideoxynucleoside activites against replication of human immunodeficiency virus. *Proc Natl Acad Sci USA* 1995; **92**: 8333-7.
47. Fontecave M, Lepoivre M, Elleingand E, Gerez C. Guittet O. Resveratrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett* 1998; **421**: 277-9.

(Received 6 October 1998; revised form accepted 22 October 1998)