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FLAVONOIDS APIGENIN AND QUERCETIN INHIBIT MELANOMA GROWTH AND METASTATIC POTENTIAL

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Flavonoids are a class of polyphenolic compounds widely distributed in the plant kingdom, which display a variety of biological activities, including chemoprevention and tumor growth inhibition. Our aim was to investigate the effects of several polyphenols on the growth and metastatic potential of B16-BL6 melanoma cells *in vivo*. Intraperitoneal administration of quercetin, apigenin, (–)-epigallocathechin-3-gallate (EGCG), resveratrol, and the anti-estrogen tamoxifen, at the time of i.m. injection of B16-BL6 cells into syngeneic mice, resulted in a significant, dose-dependent delay of tumor growth, without toxicity. The relative descending order of potency was EGCG > apigenin = quercetin = tamoxifen > resveratrol > control. Furthermore, polyphenols significantly potentiated the inhibitory effect of a non-toxic dose of cisplatin. When tested for the ability to inhibit lung colonization, quercetin, apigenin, and tamoxifen (but not EGCG or resveratrol) significantly decreased the number of B16-BL6 colonies in the lungs in a dose-dependent manner, with quercetin and apigenin being more effective than tamoxifen. Interestingly, quercetin, apigenin, and tamoxifen (but not EGCG or resveratrol) significantly decreased the invasion of B16-BL6 cells *in vitro,* with quercetin and apigenin being more effective than tamoxifen. This suggests that anti-invasive activity is one of the mechanisms underlying inhibition of lung colonization by quercetin and apigenin. In conclusion, quercetin and apigenin inhibit melanoma growth and invasive and metastatic potential; therefore, they may constitute a valuable tool in the combination therapy of metastatic melanoma. *Int. J. Cancer* 87:595–600, 2000.
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Several polyphenolic compounds are known as cancer chemopreventive agents (Kuroda and Hara, 1999). In particular, flavonoids are a class of natural polyphenolic compounds, widely distributed in the plant kingdom, that display a variety of biological activities, including tumor growth inhibition and chemoprevention (Formica and Regelson, 1995). We and others have shown that the flavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone) inhibits the growth of human mammary, ovarian, colorectal, leukemic, and lung tumor cells (Markaverich *et al.,* 1988; Scambia *et al.,* 1990b; Ranelletti *et al.,* 1992; Larocca *et al.,* 1995; Caltagirone *et al.,* 1997). We have also shown that quercetin inhibits the *in vitro* growth and clonogenesis of human melanoma cells, probably through type II estrogen-binding sites (Piantelli *et al.,* 1995). However, other mechanisms should be taken into consideration. Flavonoids inhibit the activity of several enzymes that regulate cell proliferation (Formica and Regelson, 1995), arrest cell-cycle progression (Yoshida *et al.,* 1990; Ranelletti *et al.,* 1992; Lepley *et al.,* 1996; Plaumann *et al.,* 1996), and induce apoptosis (Wei *et al.,* 1994; Plaumann *et al.,* 1996; Ahmad *et al.,* 1997). The anti-estrogen tamoxifen inhibits the proliferation of melanoma cells independently from its anti-estrogenic mechanism of action, probably by interacting with type II estrogen-binding sites (McClay *et al.,* 1993; Piantelli *et al.,* 1995). Moreover, clinical findings have indicated that addition of tamoxifen to combination chemotherapy regimens increases the response of melanoma patients (Del Prete *et al.,* 1984; McClay *et al.,* 1989; Cocconi *et al.,* 1992; Gause *et al.,* 1998). Our aim was to investigate the effects of several polyphenols, as well as of tamoxifen, on the growth and metastatic potential of the highly metastatic murine B16-BL6 melanoma *in vivo.*

MATERIAL AND METHODS

Cells

The B16-BL6 cell line is a murine melanoma variant, highly metastatic to the lungs of syngeneic C57BL/6N mice (Talmadge and Fidler, 1982), selected from the parent B16 cell line (Hart, 1979). Tumor cells were grown as monolayers in DMEM (GIBCO, Paisley, UK) supplemented with 10% heat-inactivated FCS (GIBCO) and 2 mM glutamine. For injection, cells were harvested in growth phase. High-affinity estrogen-binding sites in B16-BL6 cells were assayed using the dextran-coated charcoal method. Furthermore, the presence of estrogen receptor proteins was assayed immunohistochemically by anti-estrogen receptor antibodies (Neomarkers, Union City, CA) in cytocentrifuged preparations of B16-BL6 cells. Both methods failed to reveal these receptors. Type II estrogen-binding sites were assayed by the hydroxyapatite method, as described (Piantelli *et al.,* 1995).

Drugs and chemicals

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), apigenin (4′,5,7-trihydroxyflavone), hesperidin (3′,5,3-hydroxy-4-methoxyflavanone), rutin (3-rhamnosylglucoside of quercetin), (–)-epigallocathechin-3-gallate (EGCG; [2R,3R]-2-[3,4,5-trihydroxyphenyl]-3,4-dihydro-1[2H]-benzopyran-3,5,7-triol-3-[3,4,5-trihydroxybenzoate]), resveratrol (3,4′,5-trihydroxy-*trans*-stilbene), curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), tamoxifen (*trans*-2[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethylamine), and cisplatin (*cis*-diamminedichloroplatinum) were obtained from Sigma (Deisenhofen, Germany). Ipriflavone (isoflavone) was a gift from Chiesi (Parma, Italy).

In vitro *cell proliferation*

Cells were plated at 10^4/cm^2 in 16 mm wells of 24-well plates (Falcon, Becton Dickinson, Oxnard, CA). After 18 hr, the medium was replaced with fresh medium containing the compounds to be tested. All compounds, except EGCG, were taken from an absolute ethanol stock solution, and control cells were treated with the same amount of vehicle. The final ethanol concentration did not exceed 0.5% (v/v), either in control or in treated samples. EGCG was

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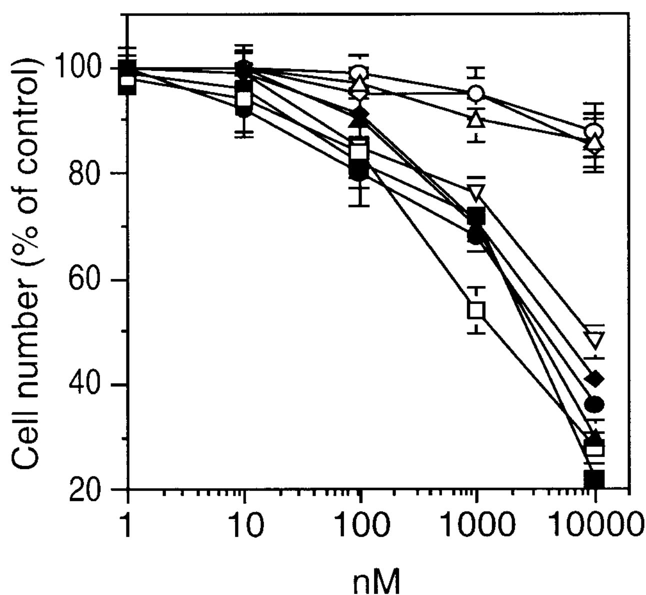

FIGURE 1 – Polyphenolic compounds inhibit the growth of B16-BL6 melanoma cells *in vitro*. Cells were plated at 10^4/cm^2 in 16 mm wells of a 24-well plate. After 18 hr, the medium was replaced with fresh medium containing the compounds to be tested. Treatments were repeated after 24 hr. Quadruplicate hemocytometric counts of triplicate cultures were performed after 72 hr of exposure to the compounds. The control value was 160,000 ± 10,000 cells/well. Closed triangles, quercetin; open squares, apigenin; closed circles, curcumin; closed squares, tamoxifen; inverted open triangles, EGCG; closed diamonds, resveratrol; open triangles, ipriflavone; open circles, rutin; open diamonds, hesperidin. Results from 3 experiments performed in triplicate are expressed as means ± SD.

TABLE I – POLYPHENOLS AND TAMOXIFEN INHIBIT MELANOMA CELL GROWTH

Treatments	IC_{50} (μM)
Apigenin	1.5
Quercetin	3.5
EGCG	8.9
Tamoxifen	2.8
Curcumin	3.6
Resveratrol	5.0

Results are expressed as mean of 3 experiments in triplicate. SDs were <10%.

dissolved in H_2O. Treatments were repeated after 24 hr. Quadruplicate hemocytometric counts of triplicate cultures were performed after 72 hr of exposure to the compounds. For thymidine-incorporation studies, cells were plated at 10^4/cm^2 in 96-well microtiter plates (Falcon, Becton Dickinson) in 10% FCS medium. After 18 hr, the medium was removed, and cells were maintained in 0.2% FCS medium for 72 hr to induce cell-cycle arrest. The medium was then replaced with fresh 10% FCS medium containing the compounds to be tested or vehicle, as described above. The level of thymidine incorporation was determined as previously described (Caltagirone *et al.,* 1997).

Tumor growth assay

Female C57BL/6N mice (6 to 8 weeks old) were obtained from Charles River (Calco, Italy). Animals were age- and weight-matched in each experiment. Tumor cells (5×10^4), in 0.1 ml PBS, were injected i.m. into the gastrocnemius muscle of mice, which were randomized into experimental groups of 6 to 8 animals receiving vehicle or daily i.p. treatments at the indicated doses. Quercetin, apigenin, resveratrol, curcumin, and tamoxifen were suspended by sonication in PBS containing 20% polyethylene glycol (PEG 400) and 2% Tween-80 (pH 7.2) (Gerritsen *et al.,* 1995). EGCG and cisplatin were dissolved in H_2O. Treatments were started on the day of tumor cell injection for all compounds except cisplatin. Cisplatin was administered i.v. 3 days after tumor cell injection at 3-day intervals for a total of 3 doses. Tumor growth was measured 3 times a week with a caliper, and tumor volume was estimated by the formula length × width^2/2 (Giavazzi *et al.,* 1986; Alessandri *et al.,* 1987; Chirivi *et al.,* 1994). The mean volume of the leg on the day of tumor cell injection was 0.05 ± 0.01 cm^3. All animals were treated according to European Community guidelines.

Lung-colonization assay

Mice were injected with 5×10^4 tumor cells in 0.1 ml PBS in the lateral tail vein. Treatments were administered i.p. daily, starting 1 hr before tumor cell injection. Mice were killed by cervical dislocation 14 to 18 days after tumor cell injection. Lungs were fixed in Bouin's solution, and black tumor cell colonies were counted by 2 different operators.

Organ-distribution assay

Cultures of B16-BL6 cells were pre-labeled with 5-[^{125}I]-iodo-2′-deoxyuridine (Amersham, Aylesbury, UK) at 0.3 mCi/ml for 24 hr. Labeled tumor cells, at a concentration of 5×10^4 in 0.1 ml PBS, were injected i.v. in mice receiving vehicle or i.p. treatment 1 hr before cell injection. At 10 min and 2, 4, 8, and 24 hr after tumor cell injection, mice were killed and the lungs removed and washed with 3 changes of 70% ethanol. Radioactivity in lungs, liver, spleen, kidneys, blood, and bladder urine was determined using a gamma-counter (Fidler, 1970)

In vitro *invasion assay*

The chemo-invasion assay was performed as previously described (Albini *et al.,* 1987). Briefly, 13 mm diameter polycarbonate filters (polyvinylpyrrolidone-free, 5 μm pore size; Nucleopore, Pleasanton, CA) coated with 15 μg/filter of basement membrane Matrigel (Becton Dickinson, Milan, Italy) were placed in Boyden chemotaxis chambers. Cells suspended at a concentration of 3×10^5/0.8 ml in DMEM were added to the upper chamber with or without the compounds to be tested. The lower chamber contained 0.2 ml of serum-free conditioned medium obtained from 18 hr cultures of 30×10^4 Swiss 3T3 cells, diluted 1:2, as a chemo-attractant. Boyden chambers were incubated at 37°C for 6 hr; then, cells on the upper surface of the filters were mechanically removed and the filters fixed in methanol and stained with hematoxylin and eosin. Cells that had invaded the lower surface of the filters were counted under a light microscope over 5 fields (magnification ×400). Each condition was tested in triplicate. B16-BL6 cell viability was greater than 90%, as assessed in parallel cultures by Trypan blue staining.

Statistical analysis

The effects of treatments on intramuscular tumor growth were evaluated by ANOVA with repeated measures. *Post hoc* comparisons were then performed by Scheffe's test, at a significance level of 5%, to compare the efficacy of treatments. Two-tailed Student's *t*-test for unpaired comparisons was used to assess the significance of the effects of treatments on the formation of tumor colonies in the lungs and on B16-BL6 invasive activity.

RESULTS

Polyphenolic compounds inhibit the growth of B16-BL6 melanoma cells in vitro

The *in vitro* growth of B16-BL6 melanoma cells in the presence of a series of polyphenolic compounds (10^{-9} to 10^{-5} M) was evaluated. Quercetin, apigenin, EGCG, resveratrol, curcumin, and tamoxifen inhibited the growth of B16-BL6 cells in a concentration-dependent manner (Fig. 1). The concentrations needed to

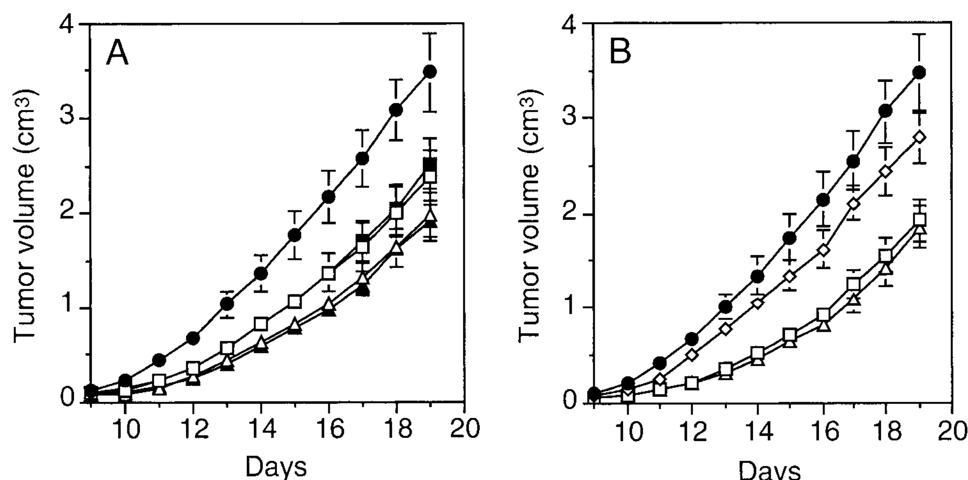

FIGURE 2 – Polyphenolic compounds inhibit the growth of B16-BL6 melanoma cells *in vivo.* B16-BL6 cells (5×10^4) were injected into the gastrocnemius muscle. Mice were treated i.p. each day with the compounds to be tested or vehicle (control). Results are expressed as means ± SD (n = 8 mice/group) and represent 3 experiments. *(a)* Closed circles, control; open squares, 25 mg/kg apigenin; open triangles, 50 mg/kg apigenin; closed squares, 25 mg/kg quercetin; closed triangles, 50 mg/kg quercetin. *(b)* Closed circles, control; open diamonds, 50 mg/kg resveratrol; open squares, 50 mg/kg tamoxifen; open triangles, 50 mg/kg EGCG. ANOVA of the data showed that both the treatments and the time of treatments (days) had a significant effect on tumor volume ($p < 0.0001$). The interaction "days × treatments" was significant ($P < 0.0001$). *Post hoc* comparisons by Scheffe's test showed that quercetin at 25 and 50 mg/kg; apigenin at 25 and 50 mg/kg; and EGCG, resveratrol, and tamoxifen at 50 mg/kg significantly delayed tumor growth ($p < 0.0001$). At 50 mg/kg, the ranking order of potency was EGCG > apigenin = quercetin = tamoxifen > resveratrol > control.

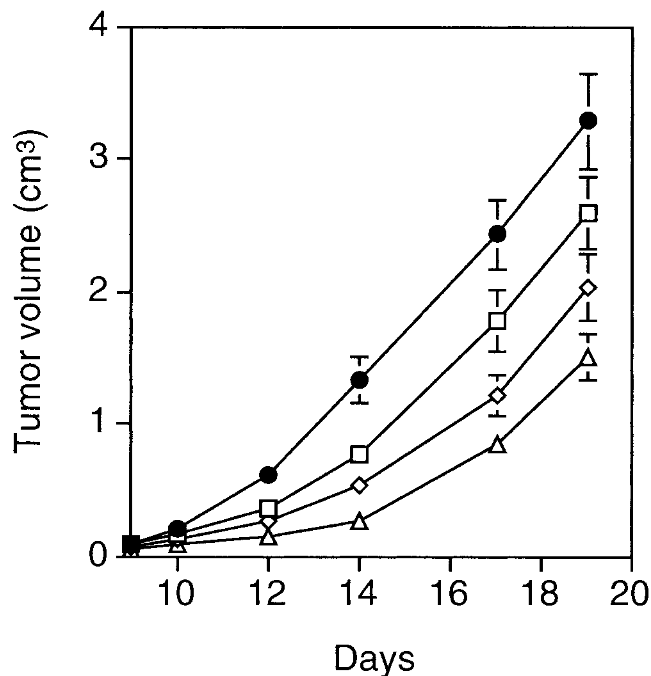

FIGURE 3 – Apigenin increases the inhibitory effect of cisplatin on B16-BL6 melanoma cell growth *in vivo.* The experiment was performed as described in Figure 2. Cisplatin was administered i.v. 3 days after tumor cell injection at 3-day intervals, for a total of 3 doses. Results are expressed as mean ± SD (n = 8 mice/group) and represent 3 experiments. Closed circles, control; open squares, 25 mg/kg apigenin; open diamonds, 2 mg/kg cisplatin; open triangles, 25 mg/kg apigenin + 2 mg/kg cisplatin. ANOVA of the data showed that both the treatments and the time of treatments (days) had a significant effect on tumor volume ($p < 0.0001$). The interaction "days × treatments" was significant ($p < 0.0001$). *Post hoc* comparisons by Scheffe's test showed that both apigenin and cisplatin alone significantly affected tumor volume ($p < 0.0001$) and that the combination of apigenin and cisplatin was significantly more effective than cisplatin or apigenin alone ($p < 0.0001$).

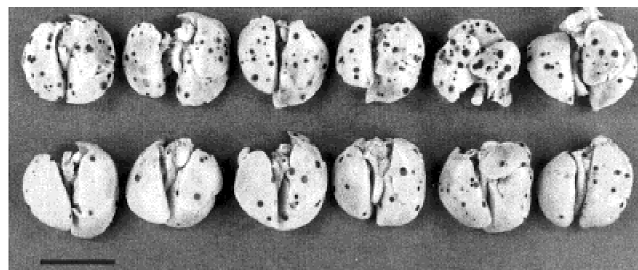

FIGURE 4 – Quercetin inhibits lung colonization of B16-BL6 cells. Cells (5×10^4) were injected in the lateral tail vein of 6 mice. Quercetin was given i.p. each day at 50 mg/kg. Lungs from mice treated with vehicle (upper row) and quercetin (lower row). Scale bar = 1 cm.

inhibit cell growth by 50% (IC_{50}) are reported in Table I. In contrast, rutin, hesperidin, and ipriflavone were ineffective (Fig. 1). The inhibitory effect of tamoxifen could not be reversed by addition of exogenous estrogens (data not shown). In agreement with this observation, we found that B16-BL6 cells were estrogen receptor-negative. The compounds that inhibited cell growth also inhibited B16-BL6 DNA synthesis, as assessed by thymidine incorporation, with IC_{50} values similar to those observed to reduce cell numbers (data not shown).

Polyphenolic compounds inhibit the growth of B16-BL6 melanoma cells in vivo

The effect of the compounds which inhibited *in vitro* cell growth was evaluated *in vivo* on i.m. melanoma growth at doses of 50, 25, and 12.5 mg/kg. No mortality or body weight changes were observed at doses up to 50 mg/kg. For all compounds, at concentrations higher than 50 mg/kg, body weight loss was observed (data not shown). In a typical experiment, tumors in control mice grew to an initial size of 1.35 ± 0.20 cm^3 (mean ± SD) within 2 weeks of the injection and then to 3.46 ± 0.41 cm^3 after 5 more days (Fig. 2). Quercetin at 25 and 50 mg/kg; apigenin at 25 and 50 mg/kg; and EGCG, resveratrol, and tamoxifen at 50 mg/kg significantly delayed tumor growth (Fig. 2*a,b*). ANOVA of the data in Figure 2 showed that the treatments and the time of treatments

TABLE II – QUERCETIN AND APIGENIN INHIBIT LUNG COLONIZATION OF B16-BL6 CELLS[1]

Experiment	Control	Quercetin (mg/kg)		Apigenin (mg/kg)		Tamoxifen (mg/kg)		EGCG (mg/kg)		Resveratrol (mg/kg)	
		25	50	25	50	25	50	25	50	25	50
1	86 ± 19[2] (100)	65 ± 12[3] (75)	29 ± 5[4] (34)	61 ± 13[3] (71)	34 ± 4[4] (40)	73 ± 15 (85)	48 ± 10[4] (56)	83 ± 18 (96)	85 ± 20 (99)	82 ± 19 (95)	74 ± 13 (86)
2	123 ± 19 (100)	93 ± 10[3] (77)	42 ± 12[4] (34)	91 ± 12[3] (74)	43 ± 5[4] (35)	105 ± 20 (85)	66 ± 9[4] (54)	121 ± 13 (98)	117 ± 14 (95)	115 ± 12 (93)	110 ± 11 (89)
3	77 ± 16 (100)	61 ± 6[3] (79)	30 ± 7[4] (39)	58 ± 8[3] (75)	34 ± 6[4] (44)	66 ± 10 (86)	42 ± 7[4] (54)	77 ± 18 (100)	72 ± 13 (93)	77 ± 15 (100)	76 ± 14 (99)

[1]Experiments were performed as described in Figure 4. Colonies were counted by 2 different operators 14 days after tumor cell injection.–[2]Results are expressed as means ± SD and % of control (in parentheses).–[3]$p < 0.05$.–[4]$p < 0.001$.

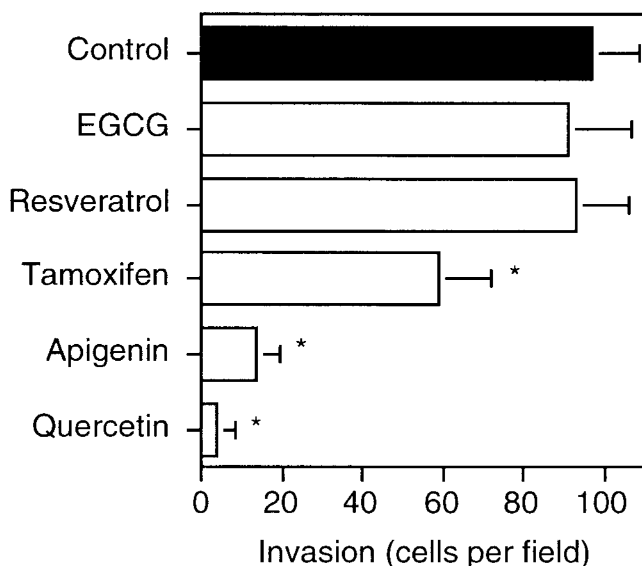

FIGURE 5 – Quercetin and apigenin inhibit invasion of B16-BL6 cells *in vitro.* B16-BL6 cells (3×10^5) were added to the upper compartment of a Boyden chamber with or without the indicated compounds (10^{-5} M). The lower chamber contained medium supplemented with 50% Swiss 3T3 supernatant, as a chemo-attractant. After 6 hr, cells that had invaded the lower surface of the filters were counted under a light microscope over 5 fields (×400). Results from 4 experiments performed in triplicate are expressed as means ± SD. *$p < 0.001$

(days) had a significant effect on tumor volume. The interaction "days × treatments" was also significant, indicating that the effects of the compounds were positively influenced by time. *Post hoc* comparisons of treatments, performed by Scheffe's test, showed that quercetin at 25 and 50 mg/kg; apigenin at 25 and 50 mg/kg; and EGCG, resveratrol, and tamoxifen at 50 mg/kg significantly delayed tumor growth. At 50 mg/kg, the relative potency of the compounds in descending order was EGCG > apigenin = quercetin = tamoxifen > resveratrol > control. At 25 mg/kg, EGCG and tamoxifen, but not resveratrol, significantly inhibited i.m. tumor growth (data not shown). At 12.5 mg/kg, all compounds were ineffective. Curcumin was ineffective at all doses tested. Since quercetin has been reported to potentiate the effect of cisplatin on ovarian and lung tumor cell growth (Scambia *et al.,* 1990*a*, 1992; Hofmann *et al.,* 1990), we administered cisplatin alone or in combination with flavonoids to mice receiving i.m. B16-BL6 cells (Fig. 3). As resulted from *post hoc* comparisons of treatments, performed by Scheffe's test, cisplatin at 2 mg/kg, the highest non-toxic dose in terms of body weight loss, significantly inhibited melanoma growth and apigenin at 25 mg/kg potentiated its effect without mortality or body weight loss. EGCG and quercetin also increased the inhibitory activity of cisplatin (data not shown).

Quercetin and apigenin potently inhibit lung colonization of B16-BL6 cells

The compounds which inhibited B16-BL6 tumor growth were tested for their ability to inhibit lung colonization. The macroscopic appearance of the lungs from untreated and treated mice clearly showed that 50 mg/kg quercetin reduced the number of lung metastases after i.v. injection of B16-BL6 cells (Fig. 4). When the effects of treatments were quantitatively evaluated, we found that quercetin and apigenin, at both 50 and 25 mg/kg, significantly decreased the number of colonies, while tamoxifen was effective only at the highest dose (Table II). In addition, in each of the 3 experiments shown in Table 2, quercetin and apigenin at 50 mg/kg were significantly more effective than tamoxifen at the same dose ($p < 0.05$). The inhibitory activities of quercetin and apigenin were similar and dose-dependent, being more effective at 50 than at 25 mg/kg ($p < 0.001$). Both compounds were ineffective at 12.5 mg/kg. EGCG and resveratrol were ineffective even at the highest doses used. The distribution of radiolabeled B16-BL6 cells in lungs, liver, spleen, kidneys, blood, and bladder urine of mice was not modified by any of the polyphenolic compounds or by tamoxifen.

Quercetin and apigenin potently inhibit invasion of B16-BL6 cells in vitro

Quercetin, apigenin, EGCG, resveratrol, and tamoxifen (10^{-5} M) were examined for their effect on invasion of B16-BL6 cells *in vitro.* Quercetin, apigenin, and tamoxifen, but not EGCG or resveratrol, significantly ($p < 0.001$) inhibited cell invasion (Fig. 5). Quercetin and apigenin were significantly ($t = 12.024$, $p < 0.001$ and $t = 9.052$, $p < 0.001$, respectively) more effective than tamoxifen, displaying similar potency with an inhibition of cell invasion of 96% and 90%, respectively.

DISCUSSION

Although flavonoids have received extensive attention because of their anti-carcinogenic activity (Formica and Regelson, 1995; Kuroda and Hara, 1999), their *in vivo* potential as anti-cancer agents has been largely unexplored (Hofmann *et al.,* 1990; Record *et al.,* 1997; Carbo *et al.,* 1999). Following previous findings by our group in human melanoma cells *in vitro* (Piantelli *et al.,* 1995), we show here that flavonoids are effective in various aspects of tumor formation in the B16-BL6 mouse melanoma model *in vivo.* This model was chosen on the basis of the aggressive behavior and high metastatic potential of this cell line (Hart, 1979; Talmadge and Fidler, 1982). Intraperitoneal administration of the flavonoids quercetin, apigenin, and EGCG, at the time of the i.m. injection of tumor cells, resulted in a significant, dose-dependent delay in tumor growth. The anti-estrogen tamoxifen inhibits B16-BL6 tumor growth with a potency similar to that of quercetin, apigenin, and EGCG. Resveratrol, a polyphenolic compound, has been identified as an anti-carcinogenic and anti-proliferative agent (Jang *et al.,* 1997; Mgbonyebi *et al.,* 1998; Carbo *et al.,* 1999; Hsieh and Wu, 1999). It is active in melanoma growth, albeit with a lower potency than the above-mentioned flavonoids. A definite assessment of the relative efficacy of these compounds, however, will

require further studies on their bioavailability and pharmacodynamics. We have observed that not all molecules that inhibit i.m. tumor growth also inhibit metastatic potential. Quercetin and apigenin administered *in vivo* reduce the number of tumor colonies in the lungs, with a similar potency, in a dose-dependent manner, whereas EGCG and resveratrol are ineffective. Cell matrix invasion is one of the phases involved in the multistep metastatic process (Westermarck and Kahari, 1999). Interestingly, quercetin and apigenin, but not EGCG or resveratrol, display potent anti-invasive activity on B16-BL6 cells *in vitro,* suggesting that the anti-invasive activity is one of the mechanisms underlying quercetin and apigenin inhibition of lung colonization. Tamoxifen reduces the metastatic potential of B16-BL6 cells *in vivo.* In agreement with previous data (Dewhurst *et al.,* 1997), we have found that it also inhibits melanoma cell invasion *in vitro.* However, in both cases, it is significantly less potent than quercetin and apigenin. The anti-tumoral effects of flavonoids and tamoxifen do not depend on the presence of estrogen receptors, which are not expressed by B16-BL6 cells, and could be mediated in part by type II estrogen-binding sites, which are expressed by these cells (data not shown). Clearly, other mechanisms could be considered. Flavonoids indeed interfere with a variety of cellular enzymes, such as tyrosine kinase, phosphatidylinositol kinase, protein kinase C, and calmodulin (Cushman *et al.,* 1991; Ferry *et al.,* 1996; Nishioka *et al.,* 1989; Singhal *et al.,* 1995; Agullo *et al.,* 1997; Darbre *et al.,* 1984; O'Brian *et al.,* 1988; Formica and Regelson, 1995) with an arrest of the cell cycle (Ranelletti *et al.,* 1992; Yoshida *et al.,* 1990; Lepley *et al.,* 1996; Plaumann *et al.,* 1996). In addition, apigenin, EGCG, and other diet-derived flavonoids (Liu *et al.,* 1996; Cao and Cao, 1999; Fotsis *et al.,* 1997) able to inhibit cell proliferation inhibit angiogenesis, which is essential for the growth of solid tumors (Folkman, 1995). Also, *in vitro* pre-treatment of melanoma cells with 20 μM genistein, a molecule which is isomeric to flavonoids, impairs the ability of these cells to colonize the lungs (Yan and Han, 1997). Interestingly, this inhibitory activity involves suppression of tyrosine phosphorylation of the focal adhesion kinase (Yan and Han, 1997). Furthermore, inhibition of calmodulin can mediate a decrease in melanoma cell invasiveness (Dewhurst *et al.,* 1997). Clearly, much more research is needed to define the mechanisms of action of polyphenols on cancer cells *in vivo.* Whatever the mechanism, flavonoids are capable of reverting the phenotype of multidrug resistance and interact in a synergistic manner with cisplatin in different tumor cell types (Scambia *et al.,* 1991; Hofmann *et al.,* 1990). Here, we have demonstrated that flavonoids co-operate positively with cisplatin in inhibiting melanoma growth *in vivo.* In conclusion, quercetin and apigenin potently inhibit melanoma growth and invasive and metastatic potential; therefore, they may constitute a valuable tool in the combination therapy of metastatic melanoma and in the prevention of melanoma metastases. These potential applications are supported by a study performed on healthy volunteers (Gugler *et al.,* 1975) and by a phase I clinical trial (Ferry *et al.,* 1996), which has shown that quercetin has low toxicity in humans.

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