Flavonoids, Dietary-derived Inhibitors of Cell Proliferation and in Vitro Angiogenesis

Theodore Fotsis, Michael S. Pepper, Erkan Aktas, Stephen Breit, Sirpa Rasku, Herman Adlercreutz, Kristina Wähläli, Roberto Montesano, and Lothar Schweigerer

Division of Hematology and Oncology, Children’s Hospital, Ruprecht-Karls University, INF 150, 69120 Heidelberg, Germany [T. F., E. A., S. B., L. S.]; Institute of Histology and Embryology, Department of Morphology, University Medical Center, 1121 Geneva 4, Switzerland [M. S. P., R. M.]; Department of Chemistry, Organic Chemistry Laboratory, P. O. Box 55, University of Helsinki, FIN-00014 Helsinki, Finland [S. R., K. W.]; Department of Clinical Chemistry, Meilahti Hospital, University of Helsinki, SF-00290 Helsinki, Finland [H. A.]

ABSTRACT

Consumption of a plant-based diet can prevent the development and progression of chronic diseases associated with extensive neovascularization, including solid malignant tumors. In previous studies, we have shown that the plant-derived isoflavonoid genistin is a potent inhibitor of cell proliferation and in vitro angiogenesis. In the present study, we report that certain structurally related flavonoids are more potent inhibitors than genistin. Indeed, 3-hydroxyflavone, 3',4'-dihydroxyflavone, 2',3'-dihydroxyflavone, fisetin, apigenin, and luteolin inhibited the proliferation of normal and tumor cells, as well as in vitro angiogenesis, at half-maximal concentrations in the low micromolar range. We have previously demonstrated that genistin concentrations in the urine of subjects consuming a plant-based diet is 30-fold higher than in subjects consuming a traditional Western diet. The wider distribution and the more abundant presence of flavonoids in the plant kingdom, together with the present results, suggest that flavonoids may contribute to the preventive effect of a plant-based diet on chronic diseases, including solid tumors.

INTRODUCTION

Angiogenesis, the generation of new capillaries from preexisting vessels, is virtually absent in the healthy adult organism in which it is restricted to a few conditions including wound healing and the formation of corpus luteum, endometrium, and placenta. These conditions of physiological angiogenesis represent ordered, tightly regulated, and self-limited processes (1). However, in certain pathological conditions, angiogenesis is dramatically enhanced and loses its self-limiting capacity (2). Although pathological angiogenesis is seen during the development and progression of many diseases, such as rheumatoid arthritis, psoriasis, and diabetic retinopathy, from a clinical perspective, probably the most important manifestation of pathological angiogenesis is that induced by solid tumors (3). Well-vascularized tumors expand both locally and by metastasis, whereas avascular tumors do not grow beyond a diameter of 1–2 mm (1, 4). Dietary factors contribute to about one-third of potentially preventable cancers (5), and the long-known preventive effect of plant-based diets on tumorigenesis and other chronic diseases is well documented (6). Breast, prostate, and endometrial cancer belong to a group of hormone-dependent cancers that, like colon cancer and coronary heart disease, are among those chronic diseases that have a lower incidence in Asia than in Western countries (7). Immigrants from Asia who maintain their traditional diet do not increase their risk of these diseases (8); however, an increased risk for these diseases accompanies a change toward a Westernized diet (9). These data indicate that certain plant-derived dietary groups might contain compounds that exert antimutagenic and antimutagenic effects, thereby offering anticancer protection to individuals consuming such diets. Identification and characterization of such compounds might provide us with additional chemotherapeutic agents for pharmacological intervention in cancer.

The idea that dietary ingested compounds could modulate proliferation of tumor cells and pathological angiogenesis appeared to us to be an important possibility meriting further investigation. If dietary compounds were to inhibit angiogenesis, this could explain, at least in part, the long-known preventive effect of plant-based diets on tumorigenesis and other chronic diseases, such as inflammation (5). In previous studies, we examined this possibility by screening the urine of human subjects consuming a diet rich in plant products for the presence of antimutagenic and antiangiogenic compounds. This work led to the identification of the isoflavonoid genistin as a potent inhibitor of cell proliferation and in vitro angiogenesis (10, 11). Further studies showed that the excretion of genistin in urine of vegetarians is 30-fold higher than that of omnivores (12–14). In the present study, we extended these observations by investigating the antimutagenic and antiangiogenic effects of flavonoids, a group of compounds that are isomeric to isoflavonoids. Flavonoid aglycones all consist of a benzene ring (A) fused with a six-member ring (B) that is isomeric to isoflavonoids. Flavonoid aglycones carry the B ring in position 3. Flavonoids are more widely distributed in the plant kingdom (15–17), rendering them a very attractive target for further studies.

MATERIALS AND METHODS

Materials and Instrumentation. All flavonoids except 2',3'-dihydroxyflavone, 3',4'-dihydroxyflavone, coumarin, and catechin were obtained from Roth Chemikalien (Karlsruhe, Germany). 2',3'-Dihydroxyflavone and 3',4'-dihydroxyflavone were synthesized as described below. The various flavonoids were prepared in 10 mM stock solutions. Apigenin, chrysin, 3',4'-dihydroxyflavone, 3',4'-dihydroxyflavone, luteolin-7-glucoside, and 3-hydroxyflavone were dissolved in ethanol. All the other compounds were dissolved in ethanol: DMSO (1:1, v/v). DMSO was obtained from Merck (Darmstadt, Germany). When stored at 4°C, stock solutions of the substances remained bioactive for more than 1 month.

1H and 13C NMR spectra were recorded on a Varian Gemini-200 FT spectrometer. Mass spectra were obtained with a JEOL JMS SX102 mass spectrometer operating at 70 eV. Samples were introduced by a direct inlet probe. The UV spectra were recorded with a Cary 5E UV-VIS-NIR spectrophotometer. Melting points were determined in open capillary tubes with an Electrothermal apparatus and are uncorrected. TLC was conducted on Merck silica gel 60 F254.
silica gel 60 F254 plates. THF was distilled over CaH₂. LiHMDS was titrated before use with 1-pyreneacetic acid (18).

Synthesis of 2',3'-Dihydroxyflavone and 3',4'-Dihydroxyflavone. 2',3'-Dihydroxyflavone was synthesized via 2',3'-dimethoxyflavone as follows. A solution of 0.81 M LiHMDS in THF (38 ml, 30.6 mmol) was added dropwise to 2-hydroxyacetophenone (0.46 ml, 3.8 mmol) in dry THF (15 ml) under argon at −78°C. The mixture was stirred at −78°C for 1 h and then at −10°C for 2 h. Methyl 2,3-dimethoxybenzoate [prepared from 2,3-dimethoxybenzoic acid with methanol and H₂SO₄ (cat.)] at room temperature; 0.75 g, 3.8 mmol) in dry THF (3 ml) was added at −78°C in one portion. The reaction was continued at −78°C for 1 h and then at room temperature for 20 h (TLC monitoring, eluent: hexane:acetone, 7:2). The reaction mixture was poured into ice water (300 ml), acidified with conc. HCl, and extracted with ethyl acetate. The extract was dried with Na₂SO₄ and evaporated. The crude product was cyclized at room temperature with 30 ml of glacial acetic acid and 1 ml of concentrated H₂SO₄ for 24 h. The precipitated product was filtered, washed with water, and dried to give 2',3'-dimethoxyflavone in 79% yield. The crude product was recrystallized from cyclohexane, m.p. 89–90°C [lit. (19) 85.5–87.5°C], UV A₅₅₀ (95% ethanol) 297 nm (log ε 4.21), 247 (4.10). The ¹H and ¹³C NMR spectra were in accord with the structure given, and will be published elsewhere: m/z 283 (19%), 282 (M⁺, 100%), 267 (4), 162 (19), 121 (50). One m MBBr₃ in CH₂Cl₂ (21 ml) was added dropwise to a stirred solution of 2',3'-dimethoxyflavone (1.20 g, 4.3 mmol) in dry CH₂Cl₂ (18 ml) under argon at room temperature. After 1 h, the mixture was poured into water (400 ml) and refluxed for 3 h. CH₂Cl₂ was distilled, and the precipitate was filtered off and washed with water. Recrystallization from 95% ethanol gave 0.87 g of ethanol (307 ian (boga 4.37), 249 (4.10).

The same culture conditions were used for human keratinocytes, a gift from Prof. Fussening [(German Cancer Research Center), DKFZ, Heidelberg, Germany). MCF-7 cells were cultured in MEM with nonessential amino acids, sodium pyruvate (1 mmol/liter), bovine insulin (10 μg/ml/liter), 10% fetal bovine serum, and antibiotics. Tumor cells from human neuroblastoma (SH-EP) and their MYCN oncogene stable transfectants (WAC 2) were a gift from Prof. M. Schwab (DKFZ) and maintained in RPMI 1640 with 10% newborn calf serum and antibiotics. In the cultures of WAC 2 cells, the antibiotic genetin (200 μg/ml) was also added for selection.

Cell Proliferation Assay. Stock cultures were trypsinized (25), cells were adjusted to a density of 5 × 10³ cells/ml (or 2 × 10⁶ cells/ml in the case of HUVE cells) in their respective media, and seeded in 1-ml aliquots into 12-well cluster dishes. After 16 h, wells received 5-ml aliquots of either solvent only, solvent containing various concentrations of the compounds to be tested, or bFGF (2.5 ng/ml), and these treatments were renewed every other day. Cells were counted at the times indicated with a Coulter particle counter (25). Unless otherwise indicated, values of cell densities represent the means of duplicate determinations that varied by less than 10% of the mean.

In Vitro Angiogenesis Assay. Three-dimensional collagen gels were prepared in 18-mm tissue culture wells as described (26). BME cells (7.5 × 10⁴ cells in a volume of 0.5 ml of a modification of MEM containing 5% donor calf serum) were seeded into each well; after 3 days, when the cells reached confluence, the medium was changed and donor calf serum was reduced to 2%. Recombinant human bFGF (10 ng/ml) plus VEGF (30 ng/ml) together with 0.1–10 μmol/liter concentrations of the flavonoids to be tested were then added. Flavonoids were added 2 h before bFGF and VEGF on the first day of treatment. Cultures were photographed after 4 days. Invasion was quantified from three randomly selected fields per well measuring 1 × 1.4 mm by measuring the total length of all cells that penetrated the underlying gel either as single cells or in the form of cell cords (27). Unless otherwise stated, results are from at least three experiments per concentration of each flavonoid (i.e., three measurements per experiment; nine measurements per concentration of each flavonoid).

RESULTS

Several flavonoids inhibit bFGF-stimulated Endothelial Cell Proliferation. To investigate the antiangiogenic effects of flavonoids, a series of flavonoid metabolites was tested with regard to their effect on the bFGF-stimulated proliferation of BBCE cells. The list of the flavonoids was selected to cover a large range of structures to facilitate the discovery of potential structure-activity relationships (Table 1). To this list, coumarin was added as a control because of its closely related chemical structure. This initial experiment showed that several flavonoid metabolites could inhibit the in vitro proliferation of BBCE at half-maximal concentrations in the lower micromolar range (Table 2). At least three flavonoids, 3',4'-dihydroxyflavone, luteolin, and 3'-hydroxyflavone, inhibited the bFGF-induced proliferation of BBCE cells (Table 2; Fig. 1) more potently than genistein (5–6 μmol/liter; Ref. 10). Therefore, the inhibitory activity of the most potent flavonoids was approximately 2–3-fold stronger than that of the isomeric genistein. Another group of flavonoids, including apigenin, fisetin, quercetin, and eriodictyol, exhibited inhibitory activity comparable to that of genistein, whereas several flavonoids had minimal or no effect on the proliferation of endothelial cells. The result was essentially the same for the most potent metabolites, when endothelial cells of different tissues and species were tested. Thus, BAE, ACE, and HUVE cells were all inhibited in a manner similar to BBCE cells (data not shown).

Tumor Cells Are Also a Target of the Antimitotic Effects of Flavonoids. Having established the antiproliferative effects of flavonoids on endothelial cells, we next investigated their antiproliferative effects on various normal and tumor cells. The rationale was 2-fold: first, to investigate the possible direct antitumor effects of flavonoids; and second, to observe whether there is any selectivity in their antimitotic activity. Towards this end, several normal and tumor cells were used, all of human origin. The normal cells included fibroblasts (HFK2) and keratinocytes (HaCaT). The tu-
The half-maximal concentration ($\mu$mol/liter) of the inhibitory effect of the various substances was tested on the in vitro proliferative activity of BBCE cells, human fibroblasts (HFK2), BBCE cells, human keratinocytes (HaCaT), breast cancer adenocarcinoma cells (MCF7), and human neuroblastoma cells (SHEP and WAC 2).

### Table 2 Antiproliferative effects of flavonoids

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Name</th>
<th>Substitution</th>
<th>$5'</th>
<th>7</th>
<th>2'</th>
<th>3'</th>
<th>4'</th>
<th>5'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavones</td>
<td>Flavone</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>5-Methoxyflavone</td>
<td>OCH$_3$</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Chrysin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>2,3-Dihydroxyflavone</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>3',4'-Dihydroxyflavone</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Apigenin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Luteolin-7-glucoside</td>
<td>OH</td>
<td>OGLC</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Flavanones</td>
<td>Hesperetin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OCH$_3$</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Eriodictyol</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Flavanons</td>
<td>3-Hydroxyflavone</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Fisetin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Myricetin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Catechin</td>
<td>Catechin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Coumarin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
</tr>
</tbody>
</table>

mor cells were also of different tissue origins: MCF7 cells were from breast adenocarcinoma, and SHEP and WAC2 cells were of neuroectodermal origin. WAC2 cells are stable transfectants of SHEP cells that express the MYCN oncogene and thus had an increased proliferative potential on the same genetic background as the SHEP cells (23). 3’,4’-Dihydroxyflavone, luteolin, and 3-hydroxyflavone were again among the most potent antiproliferative metabolites (Fig 2; Table 2). As for endothelial cells, several of the flavonoids exhibited a potent antiangiogenic activity, especially on tumor cells, whereas others had minimal or no effect. This information is particularly useful as it establishes a structure-activity relationship as discussed below.

**Flavonoids Inhibit Angiogenesis in Vitro.** To examine whether flavonoids could inhibit angiogenesis, we used an *in vitro* experimental system that recapitulates two essential components of the angiogenic process, namely extracellular matrix invasion and capillary-like tube formation (26). As shown previously, BME cells seeded on the surface of collagen gels invade the gels when exposed to bFGF (28) or VEGF (27) and form capillary-like tubes beneath the gel surface. When 10 µM concentrations of the most potent antiangiotic flavonoids...
were added along with a synergistic combination of bFGF and VEGF, they inhibited the ability of BME cells to invade the gels and generate capillary-like structures. A quantitative analysis revealed that several flavonoids inhibited bFGF/VEGF-induced invasion of BME cells to a greater extent than the same concentration of genistein (Fig. 3). A more extensive evaluation, carried out in a dose-response manner, confirmed these findings and revealed a half-maximal concentration for the inhibition of in vitro angiogenesis which closely correlates with that of the antimitotic effects (Fig. 4). The half-maximal concentration of the inhibitory effect of genistein was around 10 μM, a value that is 15-fold lower than the value that we reported in an earlier publication (10). We have now been able to confirm that the significantly higher half-maximal concentration observed in our earlier studies was due to the reduced solubility of genistein in sodium bicarbonate as compared to DMSO (data not shown).

**DISCUSSION**

The presence of phytochemicals in vegetarian diet is thought to explain, at least in part, the long-known preventive effect of plant-based diets on tumorigenesis and other chronic diseases (5). To examine this possibility, in previous studies we screened urine of human subjects consuming a diet rich in plant products for the presence of antiangiogenic and antimitotic compounds (10, 11, 29, 30). At least three urine fractions were able to inhibit the proliferation of endothelial and neuroblastoma cells. In one of the fractions, the isoflavonoid genistein was identified as an antimitotic and antiangiogenic compound (10, 29). Another of the active urine fractions contained compounds with aromatic ortho-hydroxy groups (10). Because flavonoids, the isomers of isoflavonoids, are a widely distributed group of polyphenolic compounds in the plant kingdom (15-17) and because most of them possess aromatic ortho-hydroxy groups, we were prompted to test their biological activity on proliferation of normal and tumor cells as well as their effects on in vitro angiogenesis.

In the present study, we have established that several flavonoid metabolites exhibit strong inhibitory activity on bFGF-induced proliferation of endothelial cells in vitro (Fig. 1). To the best of our
knowledge, there are no previous reports regarding the effects of flavonoids on endothelial cells, the target cells in angiogenesis. Three flavonoids, 3',4'-dihydroxyflavone, luteolin, and 3-hydroxyflavone, at half-maximal inhibitory concentrations of 1.4, 1.9, and 2.7 \( \mu \text{M} \), respectively, were more potent than the isoflavonoid genistein (5–6 \( \mu \text{M} \)). However, this difference does not warrant a conclusion that flavonoids are more potent than isoflavonoids, because apigenin, the corresponding flavonoid isomer of genistein, with hydroxylations at positions 5, 7, and 4', exhibits a comparable half-maximal inhibitory concentration (6.5 \( \mu \text{M} \)). Endothelial cells from aorta, brain, adrenal cortex, and umbilical vein were all inhibited by flavonoids in a similar fashion, which thus exhibited no specificity for any endothelial subtype.

Endothelial cells were not the only target for flavonoids. Flavonoids inhibited the proliferation of low-density cultures of various normal (HFK2 human fibroblasts and HaCaT human keratinocytes) and tumor (MCF7 human breast cancer and SHEP and WAC2 human neuroblastoma-derived) cells. Among the normal cell types, there appeared to be a greater inhibitory effect on endothelial cells than on fibroblasts and keratinocytes (Table 2), indicating a certain degree of selectivity towards endothelial cells. In contrast, the inhibitory effect of flavonoids on tumor cells was comparable to their effect on endothelial cells (Table 2). It is possible that the rapid proliferation of endothelial cells in response to bFGF stimulation accounts for the similar inhibitory profiles seen with endothelial and tumor cells. These results point to a selective targeting of rapidly proliferating cells, a conclusion that is supported by the results obtained with the isoflavonoid genistein (11).

Although antiproliferative effects of flavonoids have been reported previously (31–34), most of these studies were confined to the effects of single or few metabolites on one or two cell types. This has made it difficult to draw any conclusion regarding a potential structure-activity correlation. In the present study, a more comprehensive approach was used in the testing of a set of various flavonoid structures on several normal and tumor cells of different origin. Our results clearly show that a nonhydroxylated ring C with oxo function at position 4 and a C2-C3 double bond is required for maximal biological activity. Catechin, which lacks both the C4 oxo group and the C2-C3 double bond, is completely devoid of antiproliferative activity. Eriodictyol, the flavanone derived from reduction of the C2-C3 double bond of the flavone luteolin, is at least 3-fold weaker in inhibiting all the cells tested. The presence of a hydroxyl function in position 3, as occurs in flavonols, seems to modify the antimitotic activity in an unpredictable manner. Thus, 3-hydroxyflavone, the 3-hydroxyflavonoid flavonol derived from flavone, has antiproliferative activity which is 4-fold greater. In contrast, quercetin, the flavonol derived from luteolin, is 2.5-fold weaker in its inhibition of cell proliferation. The catechol structure alone is insufficient for inhibition of proliferation. This is clearly demonstrated by catechin, which despite the presence of aromatic vicinal hydroxyls at positions 3' and 4', does not have an antimitotic effect. Also, 3-hydroxyflavone, which has no catechol functions, has antiproliferative activity comparable to the catecholic metabolites luteolin and 3',4'-dihydroxyflavone. On the other hand, hesperetin, derived from methylation of the catechol hydroxyl at C-4' of eriodictyol, inhibits BBE cells with a half-maximal concentration of 30 \( \mu \text{M} \), which is 4-fold weaker than the antimitotic activity of the catecholic eriodictyol (7 \( \mu \text{M} \)).

Having established the inhibitory effects of flavonoids on the proliferation of endothelial cells, we next considered it important to determine whether flavonoids had additional effects on other functions of endothelial cells, which are important for angiogenesis. Angiogenesis is a complex process requiring the coordinated, sequential involvement of a number of cellular events. Indeed, formation of new capillaries begins with a localized breakdown of the basement membrane of the parent vessel, through the finely tuned elaboration of proteolytic enzymes and their inhibitors (35); this is followed by migration of endothelial cells and invasion of the surrounding matrix. The in vitro angiogenesis assay used in the present study recapitulates these early events. Thus, inhibition of in vitro angiogenesis by flavonoids represents interference with a number of early events during angiogenesis, including endothelial cell proliferation, proteolytic enzyme production, and endothelial cell migration. We have observed that the order of potency of the various flavonoids with respect to antimitotic effects and inhibition of in vitro angiogenesis does not strictly correlate. However, the requirement for a nonhydroxylated ring C with oxo function at position 4 and a C2-C3 double bond is also valid for inhibition of in vitro angiogenesis because catechin is once again totally inactive.

Flavonoids exhibit several interesting biochemical properties (36). Inhibition of tyrosine kinases (37, 38) and protein kinase C (39) are, however, of particular importance with regard to cellular processes studied in this paper. Flavonoids are competitive inhibitors with respect to the ATP binding (37) site on a variety of enzymes, a region of considerable homology among kinases. In this context, apigenin was found to inhibit both protein kinase C and TPA stimulation of the FGF receptor (40). A further important target of flavonoids appears to be 1-phosphatidylinositol kinase (41), a key enzyme in signal transduction leading to production of second messengers, inositol 1,4,5-trisphosphate, and diacylglycerol. Quercetin in breast carcinoma cells reduced phosphatidylinositol kinase and inositol 1,4,5-trisphosphate concentration within 60 min to 5 and 6%, respectively (IC50, 6 \( \mu \text{M} \)). However, no other flavonoids were tested in this study (41). Synthetic analogs of flavonoids are potent inhibitors of cdc2 (42) and cyclin-dependent kinases (43, 44), thereby inhibiting cell cycle progression. At least in the case of cdc2, the inhibition was competitive with respect to ATP binding (42). Although inhibition of kinases appears to be the most probable target, selected flavonoids have been reported to inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I (45). The strong scavenging of free radi-
cals (36) does not appear to be the mechanism of antimitotic and antiangiogenic activity of flavonoids. Indeed, myricetin and catechin, some of the strongest protectors against single-stranded breaks induced by singlet molecular oxygen (46), are devoid of antimitotic or antiangiogenic activity.

Flavonoids comprise a large group of naturally occurring low molecular weight substances that are present in fruits, vegetables, nuts, seeds, and the stems, leaves, flowers, bark, and roots of most plants, as well as tea, coffee, and wine (15–17). The average Western diet is estimated to contain approximately 23 mg/day of quercetin, kaempferol, myricetin, apigenin, and luteolin (47), the total content of all naturally occurring flavonoids not being exactly known. No information is available about the content of flavonoids in the diet of vegetarians. However, because the concentration of genistein in the urine of vegetarians in 30-fold that of omnivores (12–14), it is possible that similar values might be also anticipated for flavonoids. Development of sensitive methods for determination of flavonoids in biological materials, a task that our group has already undertaken, is expected to provide valuable information in this direction. Considering the biological functions of flavonoids presented in this and previous studies, it is quite possible that flavonoids might contribute to the preventive effects of vegetarian diet on cancer incidence and mortality. In this respect, animal experiments have already shown that flavonoids exert inhibitory effects on carcinogenicity (48, 49). Further studies should focus on the in vivo effects of flavonoids on angiogenesis and tumorigenesis models.

ACKNOWLEDGMENTS

We thank C. DiSanza and M. Guisolan for excellent technical assistance.

REFERENCES

Flavonoids, Dietary-derived Inhibitors of Cell Proliferation and
in Vitro Angiogenesis

Theodore Fotsis, Michael S. Pepper, Erkan Aktas, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/14/2916