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Relationship Between Flavonoid Structure and Inhibition of Phosphatidylinositol 3-Kinase: A Comparison with Tyrosine Kinase and Protein Kinase C Inhibition

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ABSTRACT. Depending on their structure, flavonoids display more or less potent inhibitory effects on the growth and proliferation of certain malignant cells *in vitro*, and these effects are thought to be due to inhibition of various enzymes. We investigated the inhibitory action of fourteen flavonoids of different chemical classes on phosphatidylinositol 3-kinase α (PI 3-kinase α) activity, an enzyme recently shown to play an important role in signal transduction and cell transformation. Of the fourteen flavonoids tested, myricetin was the most potent PI 3-kinase inhibitor (IC_{50} = 1.8 μM), while luteolin and apigenin were also effective inhibitors, with IC_{50} values of 8 and 12 μM, respectively. Fisetin and quercetin, as previously reported, were also found to significantly inhibit PI 3-kinase activity. The same flavonoids were also analyzed for inhibition of epidermal growth factor receptor (EGF-R), intrinsic tyrosine kinase and bovine brain protein kinase C (PKC). At elevated doses, some of these flavonoids were found to also cause significant inhibition of PKC and tyrosine kinase activity of EGF-R. A structure-activity study indicated that the position, number and substitution of the hydroxyl group of the B ring, and saturation of the C2-C3 bond are important factors affecting flavonoid inhibition of PI 3-kinase. They may also play a significant role in specificity of inhibition and could help to provide a basis for the further design of specific inhibitors of this lipid kinase. Finally, possible relationships between the antitumoral properties of these flavonoids and their biological activities are discussed. BIOCHEM PHARMACOL **53**;11:1649–1657, 1997.

KEY WORDS. flavonoids; structure-activity study; myricetin; PI 3-kinase α; protein kinase C; EGF receptor tyrosine kinase

Flavonoids are polyphenolic compounds naturally present in plants. They exhibit a variety of effects including inhibition of malignant cell growth (for a review see [1]). Several recent studies have demonstrated that, depending on their structure, flavonoids may be potent inhibitors of several kinases involved in signal transduction, mainly protein kinase C (PKC) and tyrosine kinases. Most of these effects are directed toward the ATP-binding site of the kinase, although other still unknown mechanisms may exist [1]. Therefore, naturally occurring flavonoids have been proposed to exert biological effects on cells through inhibition of these different key enzymes. For these reasons, they may be considered as potential compounds for selectively blocking signal transduction pathways and for designing more potent analogues for use in proliferative disease therapy. In order to investigate molecular specificity, several authors have tested the effects of various flavonoids on different tyrosine kinases [2, 3] and on PKC [4]. They have shown that the position of the hydroxyl group on the phenyl ring strongly influences the conformation of the molecule and modulates their inhibitory effect.

In this study, we compared the effects of fourteen different flavonoids on another important enzyme involved in signal transduction and cell transformation: phosphatidylinositol 3-kinase α (PI 3-kinase α). To date, two highly homologous PI 3-kinases (α and β) have been described as heterodimers consisting of an 85 kDa regulatory subunit (p85 α and β) and a 110 kDa catalytic domain (p110 α and β) [5]. A third member which appears to function as a monomeric protein, i.e., p110γ, has recently been cloned [6]. It is noteworthy that the kinase domain of all the PI

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Abbreviations: DAG-kinase, diacylglycerol-kinase; EGF-R, epidermal growth factor receptor; MARCKS, myristoylated alanine rich C kinase substrate; PtdIns (3,4) P2, phosphatidylinositol 3,4 bisphosphate; PtdIns (3,4,5) P3, phosphatidylinositol 3,4,5 trisphosphate; PI 3-kinase α, phosphatidylinositol 3-kinase α; PKC, protein kinase C; TLC, thin layer chromatography.

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3-kinases described are highly homologous. PI 3-kinase α has been shown to be activated by a number of growth factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and insulin [5, 7]. In mammalian cells, agonist-dependent PI 3-kinase activation leads to rapid and transient production of phosphatidylinositol 3,4 bisphosphate (PtdIns (3,4)P2) and phosphatidylinositol 3,4,5 trisphosphate (PtdIns (3,4,5) P3) [5, 7]. These novel phosphoinositides, which are not substrates for any known phospholipase C, have been proposed to act as second messengers [5, 7]. Although their direct targets remain to be identified, they have been proposed to play an important role in mitogenesis [5, 7]. Several reports have shown that PI 3-kinase is involved in oncogenic-dependent cell transformation [8], and elevated levels of PI 3-kinase activity and PI 3-kinase products have been found to correlate with cell transformation by several oncogenes [8]. Recently, wortmannin, a fungal metabolite that exhibits strong PI 3-kinase inhibitory action at nanomolar concentrations, was shown to selectively inhibit the proliferation of bcr/abl-oncogenic tyrosine kinase-dependent chronic myelogenous leukemia cells in a mixture of normal bone marrow and Philadelphia chromosome-positive cells [9]. At higher doses, however, wortmannin has also been shown to block other enzymatic activities [10]. Therefore, the development of natural PI 3-kinase inhibitors as antitumor agents would be of great interest to design analogues that would be helpful in preventing proliferative disorders such as restenosis and various cancers. Flavonoids represent a potentially interesting family of natural compounds that may provide a basis for the screening of inhibitors of PI 3-kinase activity [11, 12]. To our knowledge, only a few flavonoids have been reported to inhibit kinases involved in the phosphoinositide metabolism, i.e., phosphatidylinositol 4-kinase and PI 3-kinase [11, 12, 13]. Using quercetin as a model, Vlahos *et al.* [14] synthetised a chromome, the LY294002, now widely used as a specific inhibitor of PI 3-kinase. In order to determine whether other flavonoids have inhibitory effects on PI 3-kinase, we tested fourteen different flavonoids from different chemical classes. These effects were compared to those observed on epidermal growth factor receptor (EGF-R) tyrosine kinase activity both *in vitro* and *in vivo*, diacylglycerol-dependent PKC activity on myristoylated alanine rich C kinase substrate (MARCKS) phosphorylation *in vitro* and their previously observed effect on tumour cell proliferation [1]. Our results reveal a structure-activity relationship that may help in the further design of analogues displaying antiproliferative effects and provide evidence for a correlation between the antitumoral properties of flavonoids and their efficiency in inhibiting signalling enzymes.

MATERIALS AND METHODS

Drugs and Chemicals

Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were obtained from Gibco BRL (Eragny, France). Flavonoids were from Sigma Chemical Co (St. Louis, MO, USA), except for luteolin, chrysin, fisetin, galangin and diosmetin, which were from Extrasynthèse (Lyon, France). All other chemicals were purchased from Sigma (St. Louis, MO, USA) and were of the highest purity available.

Cell Culture

A431 cells (a carcinoma cell line overexpressing EGF receptor) were grown in Dulbecco's modified Eagle's medium supplemented with 7.5% fecal calf serum (FCS) in a 5% CO_2 humidified atmosphere at 37°C as described previously [15].

PI 3-kinase Assay

PI 3-kinase α was isolated from human blood platelets by immunoprecipitation using an anti-p85α antibody as previously described [15]. PI 3-kinase activity was measured using immunopurified enzyme and sonicated phosphatidylinositol (250 μM) as a substrate in 100 μL containing 50 mM Tris-HCl (pH 7.3), 5 mM $MgCl_2$, 50 μM ATP, 1 mM EDTA, 2 mM dithiothreitol (DTT), 100 mM NaCl and 5 μCi [γ^{32}-P] ATP. Incubation was carried out at 37°C for 10 min and stopped by addition of two volumes of a mixture of chloroform/methanol (v/v). Lipids were immediately extracted following a modified method of Bligh and Dyer as described [15, 16]. Under these conditions, more than 98% of ^{32}P-radiolabelled products obtained were PtdIns(3)P as assessed by thin layer chromatography (TLC) and HPLC technique as described [15, 16]. Under all conditions, PtdIns(3)P formation was linear with protein concentration and with time for at least 15 min. The PtdIns(3)P produced was separated by TLC using chloroform, methanol, 4.3 M NH_4OH (90/70/20) as a solvent [15]. ^{32}P-labelled PtdIns(3)P was detected after autoradiography, recovered by scraping and the radioactivity was quantified. Alternatively the HPLC technique was used [15, 16].

In one experiment, the effect of 5 μM of myricetin was tested on the PI 3-kinase activity recovered in an antiphosphotyrosine immunoprecipitate obtained from EGF-stimulated A431 cells as previously described [15].

PI 4-kinase and Diacylglycerol-kinase Assay

These kinase activities have been shown to relocalize to the cytoskeleton of thrombin-activated platelets [17]. Therefore, we assayed these kinases in such a fraction as previously described [17], in the absence or presence of 5 μM of myricetin and using the HPLC technique [15, 16, 17] to discriminate between the different lipid kinase activities.

PKC Assay

PKC as well as MARCKS were purified from bovine brain as described [18]. The phosphorylation assays contained 20

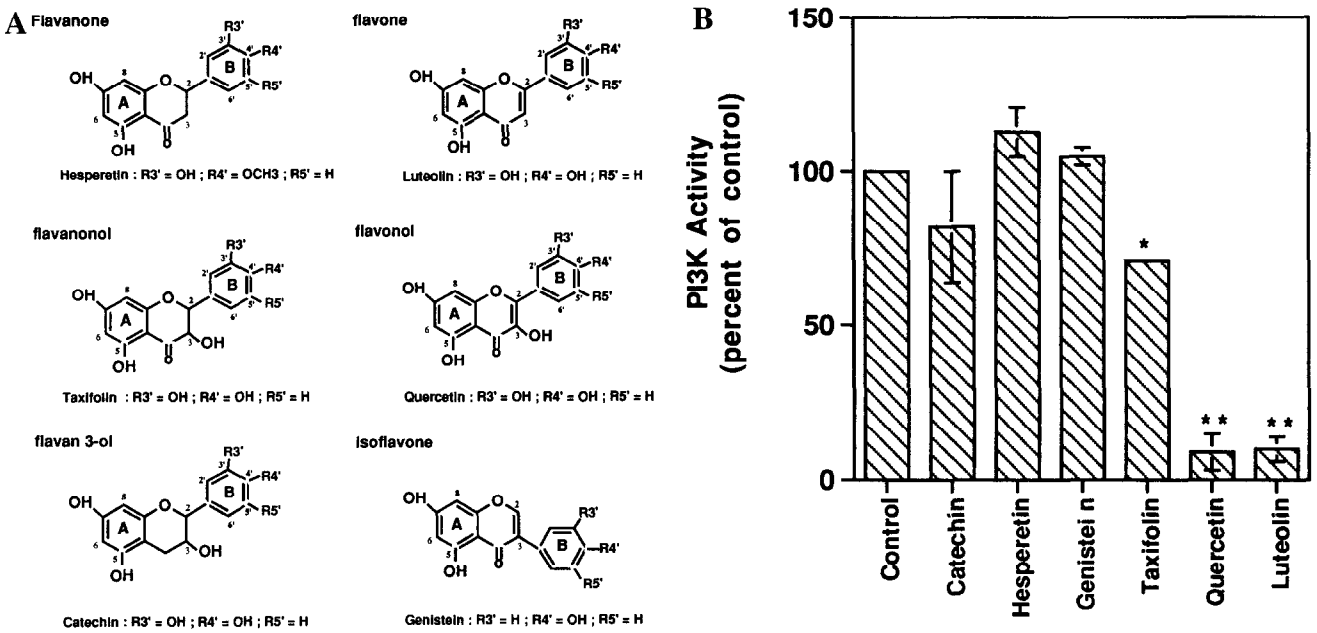

FIG. 1. Structures of the different chemical classes of flavonoids used in this study (A) and their effects on PI 3-kinase activity (B). Effects of the six different flavonoids (60 μM) on immunopurified PI 3-kinase activity were determined. Enzymatic assays were carried out as indicated in Materials and Methods. Values are expressed as the mean percentage of control PI 3-kinase activity ± SEM of three to five independent experiments using different enzymatic preparations (*$P < 0.05$; **$P < 0.01$).

mM Tris HCl (pH 7.5), 10 mM $MgCl_2$, 50 μM $CaCl_2$, 80 μg/mL phosphatidylserine, 8 μg/mL diolein, 1 ng of purified PKC and 120 ng of pure MARCKS in a total volume of 40 μL. Indicated amounts of flavonoids or DMSO in the control were added. Reactions were initiated by addition of 250 μM [γ-^{32}P]ATP and were performed at 25°C for 15 min. Reactions were stopped by addition of SDS-sample buffer, and SDS-PAGE was immediately performed according to Laemmli [19]. The gels were dried and ^{32}P-radiolabelled MARCKS was detected by using a Phosphorimager 445 SI (Molecular Dynamics, Inc, Paris, France). Under these assay conditions, the phosphorylation of MARCKS was linear with protein concentration and with time for approximatively 30 min.

EGF Tyrosine Kinase Assay

A431 cells were grown to near confluency in culture dishes and incubated overnight in serum-free medium at 37°C to reduce basal activity. Thereafter, they were pre-treated with the indicated amounts of flavonoids or a corresponding volume of DMSO (control) for 10 min and subsequently stimulated with 50 ng/mL of EGF for 5 min at 37°C. Cells were then washed with ice-cold PBS and scraped off the culture flask in ice-cold lysis buffer containing 50 mM Tris-HCl (pH 7.4), 2 mM EGTA, 10 mM EDTA, 100 mM NaF, 1 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 2 μg/mL leupeptine, 2 μg/mL aprotinin and 0.4% (v/v) Triton X-100. Homogenates were then centrifuged at 12000 × g for 10 min at 4°C and the triton X-100-soluble fractions collected. Protein concentration was determined according to the Bio-Rad Protein Assay Kit (Bio-Rad GmbH, Munich, Germany) and adjusted in each sample. They were then resuspended in SDS-sample buffer, separated by a 7.5% SDS-PAGE, transferred onto nitrocellulose and probed with the antiphosphotyrosine antibody 4G10 (obtained from Upstate, Biotechnology, Inc (New York, NY, USA), dilution 1:1000) as previously described [14]. Immunodetection was performed with peroxidase-conjugated secondary antibody using the ECL chemiluminescence system (Amersham, Buckinghamshire, UK).

Alternatively, a fraction enriched in plasma membrane vesicles was prepared from A431 cells as described [20]. The phosphorylation reaction was started by adding 100 ng/mL of EGF, 4 mM $MnCl_2$, 100 μM Na_3VO_4, 5 μM ATP in the presence or absence of flavonoids for 10 min at 20°C. The reaction was stopped by boiling in SDS-sample buffer and the tyrosine phosphorylation status of the EGF-receptor was assessed by Western blotting as described above.

RESULTS

Six representative chemical classes of flavonoids (shown in Fig. 1A) were tested on PI 3-kinase activity at a final concentration of 60 μM. As shown in Fig. 1B, the flavonol quercetin and the flavone luteolin were the most potent, inhibiting PI 3-kinase activity by approximatively 90%. The flavanonol taxifolin inhibited PI 3-kinase activity to a lesser extent and the remaining three classes of flavonoids tested, i.e., isoflavone (genistein), flavan-3-ol (catechin) and flavanone (hesperetin), did not significantly affect this enzymatic activity.

In order to gain insight into the influence of polyhydroxylation and methylation of flavonols and flavone, we

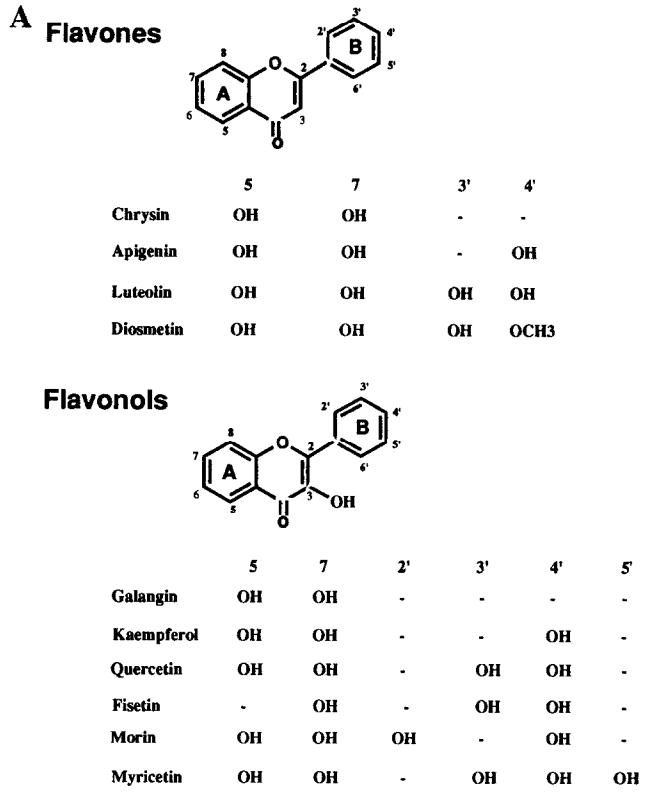

FIG. 2. Structures of six flavonols and four flavones used in this study (A) and the effect of certain of these on PI 3-kinase activity (B). The use of these flavonoids allowed us to examine the effect of the poly-hydroxylation of flavonols and flavones and the effect of the methylation of flavones. The eight different flavonoids were used at a concentration of 60 μM and their effect on PI 3-kinase activity was determined as described in Figure 1B. Values are expressed as the mean percentage of control PI 3-kinase activity ± SEM of three to five independent experiments using different enzymatic preparations (*P < 0.05; **P < 0.01).

compared the inhibitory effects of myricetin, quercetin, kaempferol and galangin and those of luteolin, apigenin, chrysin and diosmetin (Fig. 2). The chemical structures of these compounds are shown in Fig. 2A. Our results (Fig. 2B) indicate that the inhibitory effect of flavonols on PI 3-kinase activity was dependent on the number and the position of hydroxyl residues. Indeed, the most hydroxylated flavonol (myricetin) was the most potent inhibitor, whereas the less hydroxylated flavonol (galangin) induced a very weak inhibition of PI 3-kinase activity. Interestingly, morin, which bears two hydroxyl groups in the 2',4' position of the B ring, had no significant inhibitory effect (8% ± 3 at 60 μM), whereas fisetin, which is identical to quercetin but lacks the hydroxyl group in the 5 position of the A ring, had approximatively the same inhibitory effect as quercetin (80% ± 5 at 60 μM). This structure-activity relationship was also observed among the flavones, since luteolin had a more potent inhibitory effect than apigenin and chrysin had almost no effect. Diosmetin, which has a methyl substitute at the 4' position of the B ring, inhibited PI 3-kinase by only 55% ± 6, whereas luteolin had an inhibitory effect of 90% ± 4. This suggests that methylation of the 4'OH of the B ring leads to decreased inhibitory activity.

Fig. 3A summarizes the effects of ten representative flavonoids on PI 3-kinase activity. To quantify the effect of the most potent inhibitors (i.e., myricetin, quercetin, luteolin and apigenin), we measured their effects upon increasing concentrations (Fig. 3B). We found myricetin to be the most potent PI 3-kinase inhibitor with an IC_{50} of 1.8 μM, whereas luteolin, quercetin and apigenin exhibited an IC_{50} of 8, 10 and 12 μM, respectively. The differences in the assay conditions as well as in the source and the PI 3-kinase α purification protocol may explain the higher IC_{50} found for quercetin compared to that previously reported by Matter *et al.* [11].

Quercetin has been shown to compete with ATP on the PI 3-kinase catalytic domain [11], and our preliminary data indicated that myrecitin, luteolin and apigenin competed toward ATP as well (not shown).

In order to establish whether our findings would hold true for other protein kinases, we tested the effect of the above ten flavonoids on the activity of the EGF-receptor tyrosine kinase on isolated membranes and in whole A431 cells, and on the activity of purified bovine brain protein kinase C. The results, shown in Fig. 4A and B, indicate that the flavonoids did not display the same order of inhibitory potency on tyrosine kinase activity as that seen for the PI 3-kinase. Indeed, only genistein [21] and kaempferol were potent inhibitors, whereas myricetin and diosmetin were less efficient. The remaining six flavonoids (apigenin, luteolin, quercetin, hesperetin, catechin and taxifolin) did

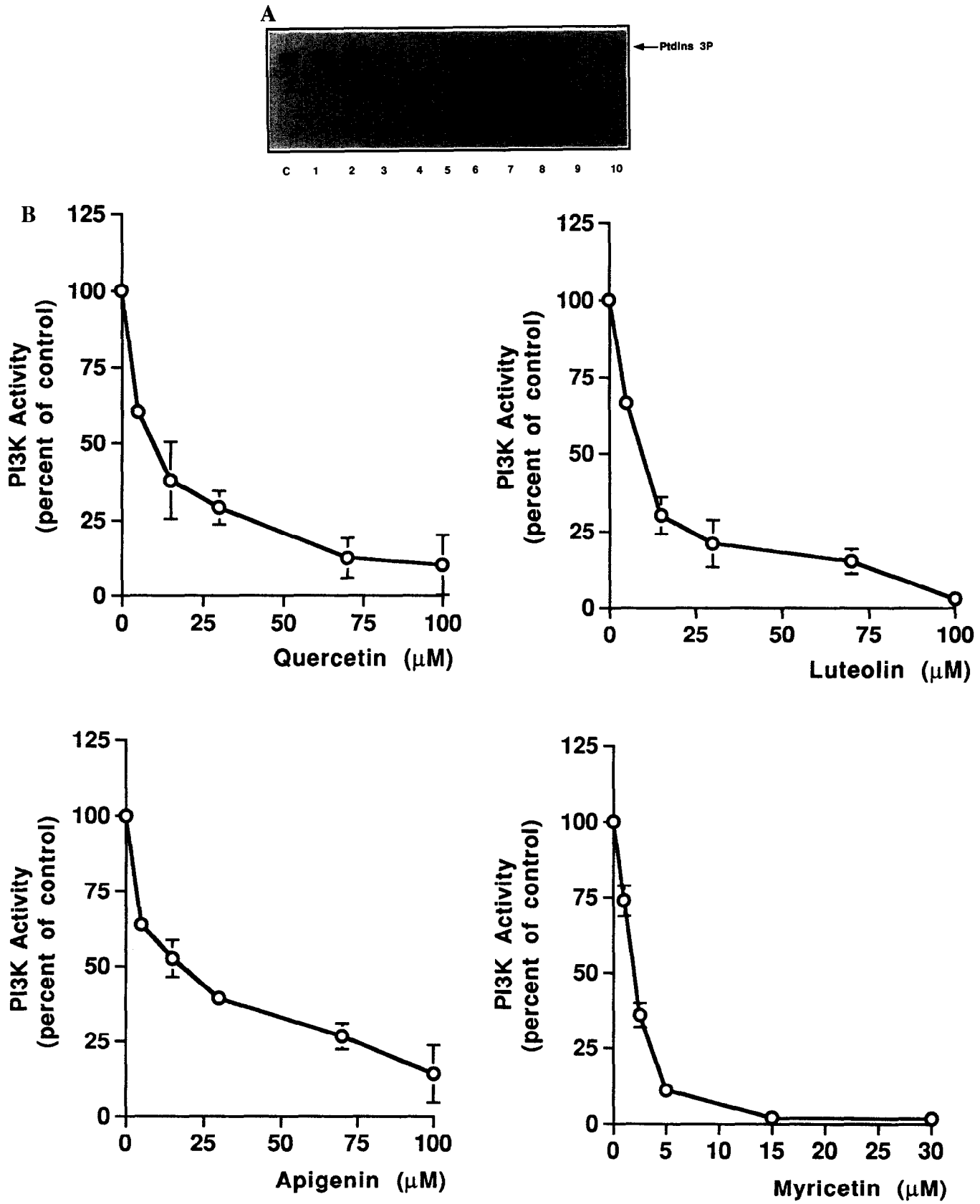

FIG. 3. Recapitulative illustration of the effects of ten representative flavonoids (A) and dose-dependent responses of flavonols and flavones on PI 3-kinase activity (B). (A) A representative autoradiography of a TLC illustrating the effect of flavonoids (60 μM) on the capacity of immunopurified PI 3-kinase to phosphorylate its substrate PtdIns into PtdIns(3)P *in vitro*. C: positive control (no flavonoid), 1: catechin, 2: hesperetin, 3: quercetin, 4: luteolin, 5: apigenin, 6: genistein, 7: kaempferol, 8: taxifolin, 9: myricetin, 10: diosmetin. (B) Dose-dependent inhibition of PI 3-kinase activity by luteolin, apigenin, quercetin and myricetin. PI 3-kinase assays were carried out as described in Materials and Methods. Values are given as the mean percentage of control PI 3-kinase activity ± SEM of three independent experiments using different enzymatic preparations. When they do not appear, error bars are smaller than the symbol size.

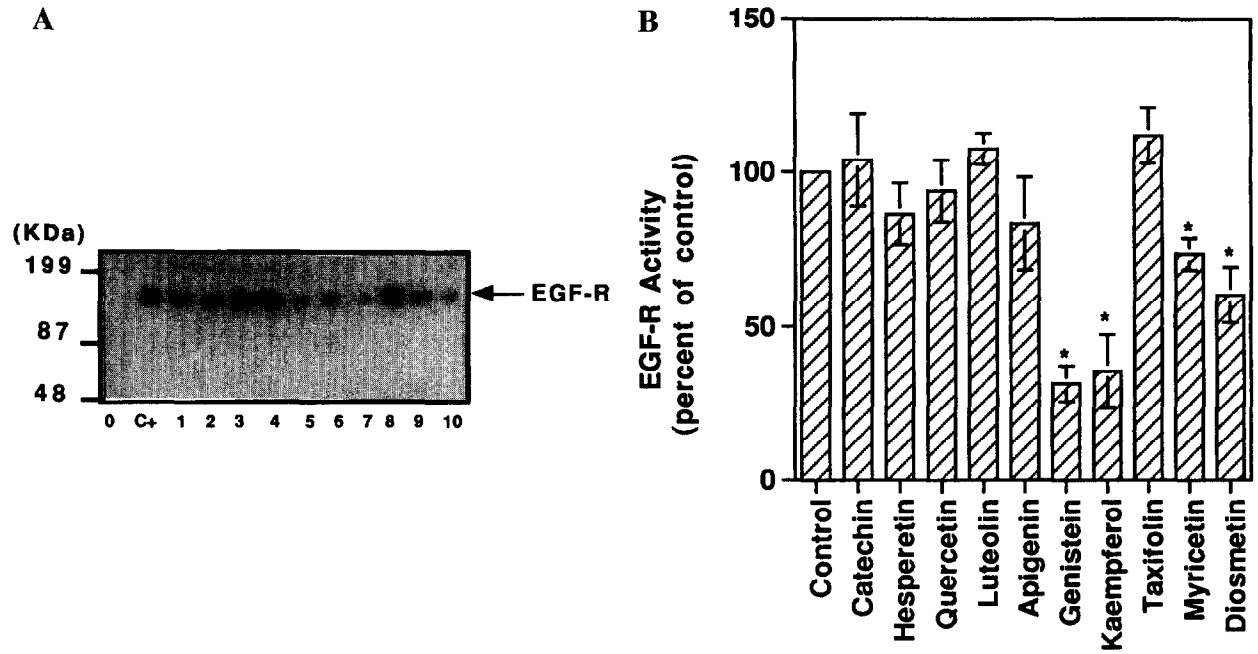

FIG. 4. Effects of the ten flavonoids on EGF-receptor intrinsic tyrosine kinase activity in EGF-stimulated A431 cells. (A) Analysis of EGF-receptor tyrosine phosphorylation as a reflection of its EGF-dependent intrinsic tyrosine kinase activity was carried out by Western blotting experiments. A431 cells were both untreated (0 and C^+) or treated (1 to 10) with 60 μM of the different flavonoids for 10 min, activated (C^+) or not (0) with 50 ng/mL of EGF for 5 min and lysed as described in Materials and Methods. Tyrosine phosphorylation of the EGF-receptor was then analyzed by Western blotting using the anti-phosphotyrosine antibody 4G10. 0: control (no EGF, no flavonoid); C+: EGF; 1: catechin, 2: hesperetin, 3: quercetin, 4: luteolin, 5: apigenin, 6: genistein, 7: kaempferol, 8: taxifolin, 9: myricetin, 10: diosmetin. (B) Quantification of the phosphorylation was performed by densitometric analysis (ScanMaker IIHR, Microtek, Germany) of the Western blot. Data are expressed as the mean ± SEM of the percentage of maximal phosphorylation observed with 50 ng/mL EGF as determined by three independent experiments (*$P < 0.05$).

not exhibit significant inhibitory effects on this tyrosine kinase *in vivo*. Similar data were obtained in an *in vitro* assay using isolated A431 cell membranes, except that genistein had an even more potent effect and quercetin displayed some EGF-receptor tyrosine kinase inhibitory action, as previously reported [21].

The effect of flavonoids on the capacity of bovine brain PKC to phosphorylate one of its physiological cellular substrate MARCKS was also tested. As shown in Fig. 5, only flavones and flavonols exhibited strong inhibitory potency, at 60 μM, on diolein-dependent protein kinase C activity, as also observed with respect to PI 3-kinase activity. The order of potency was rather similar to that found with PI 3-kinase, i.e., myricetin > luteolin > quercetin > apigenin ~ diosmetin.

Finally, the inhibitory effect of 5 μM of myricetin on PI 3-kinase α was compared to the effect of this flavonoid on diacylglycerol-kinase (DAG-kinase), PI 4-kinase, PKC and on the PI 3-kinase activity recovered in an antiphosphotyrosine immunoprecipitate obtained from EGF-stimulated A431 cells (Fig. 6). The relatively low dose of myricetin used was sufficient to strongly inhibit PI 3-kinase α (91% ± 0.5) as well as the PI 3-kinase recovered in the antiphosphotyrosine immunoprecipitate obtained from EGF-stimulated cells, but was only weakly effective on DAG-kinase and PI 4-kinase. However, PKC was still significantly inhibited (45% ± 3) by this dose of myricetin although to a lesser extent.

DISCUSSION

Flavonoids are naturally occurring plant polyphenols found in abundance in diets rich in fruit, vegetables and plant-derived beverages such as tea. These compounds are known as growth inhibitors for a variety of cancerous cell lines [1, 22–29]. Cellular mechanisms underlying these effects are still unclear but are thought to be linked to inhibition of enzymes involved in transduction of mitogenic signals [1]. Indeed, depending on their structure, some flavonoids inhibit tyrosine kinase and serine/threonine kinase activities. Genistein, an isoflavone, is a widely used inhibitor specific for tyrosine protein kinases [21], whereas the flavonol quercetin lacks selectivity since its inhibitory properties extend to various protein serine/threonine kinases and lipid kinases [30–32]. The discovery of naturally occurring inhibitors can provide interesting information regarding the type of small molecules that interact with and inhibit kinases. Such inhibitors may be useful tools for elucidating cellular processes that are mediated by growth factors and hormone-dependent serine/threonine or tyrosine phosphorylation [33].

It has previously been shown by different groups [1, 24–27] that quercetin and genistein are potent inhibitors of human carcinoma cell growth. In order to investigate the possible relationship between the antitumoral properties of some flavonoids and their biological activities at the cellular level, we have compared the effects of ten different

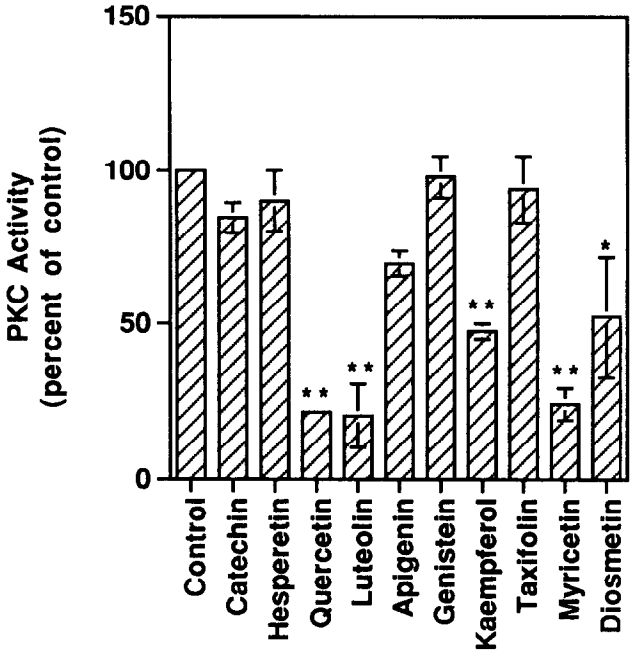

FIG. 5. Effects of the ten flavonoids on *in vitro* bovine brain PKC activity. Effects of the different flavonoids (60 μM) on bovine brain purified PKC were determined as indicated in Materials and Methods. Diolein was used as enzyme activator and purified MARCKS as a physiologic substrate. After phosphorylation of MARCKS by PKC, the fractions were submitted to a 7.5% SDS-PAGE and the effects of the ten flavonoids were analyzed by autoradiography of the gel or phosphorimager 445 SI (Molecular Dynamics, Inc). Densitometric analysis (ScanMaker IIHR, Microtek, Germany) of the autoradiogram was performed in order to quantify the phosphorylation state of MARCKS and thereby PKC activity in the presence of the different flavonoids. Data are expressed as percentage of maximal phosphorylation observed without flavonoids (control) and are mean activity ± SEM of three independent experiments (*$P < 0.05$; **$P < 0.01$).

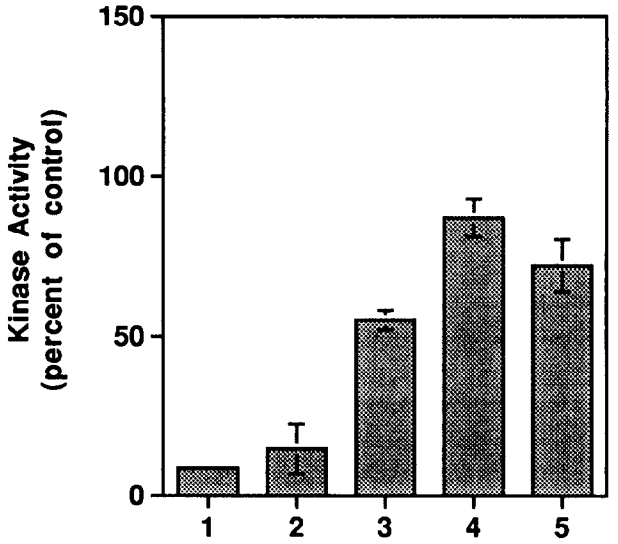

FIG. 6. Comparison of the effect of 5 μM of myricetin on various kinase activities. The effect of a relatively low dose of myricetin (5 μM) on PI 3-kinase α (1) was compared to its effect on PI 3-kinase activity recovered in antiphosphotyrosine immunoprecipitate obtained from EGF-stimulated A431 cells (2), purified brain PKC (3), DAG-kinase (4) and PI 4-kinase (5). These enzymes were obtained and assayed as indicated in Materials and Methods. Results are expressed as the percentage of maximal activity observed without flavonoid (control) and are the mean ± SEM of two to three independent experiments.

flavonoids on PI 3-kinase, on EGF-receptor tyrosine kinase and on protein kinase C activities. PI 3-kinase has recently been shown to be an important effector of the polyphosphoinositide pathway and a key enzyme involved in signal transduction and cell transformation [5, 7, 8]. The development of natural PI 3-kinase inhibitors as antitumor agents would be helpful in designing analogues that could be used for the treatment of proliferative diseases [14] and in suggesting new interpretations of the data obtained by epidemiologists that may contribute speculation about appropriate diets.

In this study, we demonstrate that PI 3-kinase is inhibited by some flavonoids in a concentration-dependent manner, depending on flavonoid structure. The most potent inhibitors of PI 3-kinase activity are flavonols and flavones. Flavan-3-ol, flavanone and isoflavone are without effect, even at the highest concentration tested (60 μM). Our results show that beyond the flavonol family, myricetin is the most potent inhibitor, with an IC_{50} of 1.8 μM. Interestingly, preliminary data indicate that myricetin (10 μM) was able to significantly inhibit the well-described [16] thrombin-dependent accumulation of $PI(3,4)P_2$ in human blood platelets† and we are currently testing its effect on the recently described PI 3-kinase α-dependent bacterial invasion [34]. However, at higher concentrations this flavonoid displays inhibitory action on other kinases such as PKC and EGF-R tyrosine kinase activity. Moreover, it has previously been shown that myricetin was able to inhibit the tyrosine kinase activity of pp130fps and the insulin receptor in the micromolar range, whereas higher doses were necessary to affect protein kinase A activity [35].

Here, we show that 5 μM of myricetin strongly inhibits PI 3-kinase whereas PI 4-kinase and DAG-kinase are weakly affected. Although this concentration is still effective on PKC (45% of inhibition), our results suggest that myricetin may provide an interesting structural model for the design of new pharmacological tools.

From our results, it is clear that the major structural requirements for the potent inhibition of PI 3-kinase by flavonoids are the presence of a 2-double bond on the C ring and the absence of substitution of hydroxyl groups (i.e., flavonols and flavones). Beyond these 2 structural classes, the most potent inhibitors have a 3′,4′ OH group on the B ring. Elimination of the 3′ OH substitute reduces the inhibitory potency within these chemical classes, as shown by comparing apigenin to luteolin and kaempferol or morin to quercetin.

Absence of the 5 OH substitute on the A ring of flavonol does not modify their inhibitory potency on PI 3-kinase

† BP, personal communication.

activity as shown by comparing fisetin to quercetin. The introduction of OH groups on the flavon ring increases the inhibitory potency of flavonoids, the polyhydroxylated flavonol myricetin being by far the most efficient. The replacement of hydroxyl groups with methoxyl substituents yielded much weaker inhibitors (diosmetin), suggesting the importance of hydrogen bonds between flavonoids and the kinase. The presence of other substituents at the 3 position (isoflavone:genistein) eliminates inhibitory activity. Saturation of the C2-C3 bond at the pyrone ring of flavonols and flavones yields flavanonols and flavanone (taxifolin and hesperetin) which were less effective and ineffective as PI 3-kinase inhibitors, respectively.

As already described by others [1, 4, 35], our results show that flavonols and flavones are also the most active inhibitors of protein kinase C, indicating that the inhibitory effect of these structural classes of flavonoids is not restricted to one type of protein kinase. In contrast, these potent flavonoids exhibit only weak inhibition of protein tyrosine kinase activity, even at the highest concentration tested. As previously shown [21], genistein displays a potent inhibitory property whereas kaempferol and hesperetin decrease this enzymatic activity to a lesser extent. It is noteworthy that genistein, which is widely used as a specific tyrosine kinase inhibitor, does not affect PKC activity or PI 3-kinase [14].

Since flavonols and flavones display antitumoral properties [22–29], one can suggest that this property is linked to the inhibition of both PI 3-kinase and protein kinase C. However, flavonoids are known to affect other enzymes such as topoisomerases [1, 36], and it is possible that their antitumoral effect is also at least in part linked to their inhibitory effect on these enzyme activities.

Since it has already been shown that flavonoids exert preferential effects on actively dividing cells [1, 28], it is tempting to speculate that these properties are linked to their inhibition of PI 3-kinase, a key enzyme involved in cell multiplication and transformation [7, 10]. In this respect, it is interesting to note that quercetin induced a specific G1 arrest in human gastric, leukemic and colonic cancer cells [24, 25, 37] as wortmannin, a potent PI 3-kinase inhibitor at nanomolar range, does in other cells. Moreover, wortmannin has recently been shown to selectively eliminate leukemia-blast crisis cells from a mixture of normal bone marrow and Philadelphia chromosome-positive cells [9]. It would be of interest to examine whether a specific target of flavonoids is needed to obtain the inhibitory effect on a specific tumour cell growth. In this respect, experiments are now in progress to investigate a possible relationship between the effects of specific inhibitors of PI 3-kinase (wortmanin at nanomolar concentration, or LY294002) or PKC (GF109209X) and those of certain flavonoids on tumour gastrointestinal cell growth. Since flavonoids, whose total consumption is likely to be as high as 100 mg per day [38], are potential anticancer agents, it is tempting to speculate that an appropriate diet may have some effect on preventing malignant tumours of the gastrointestinal tract.

Finally, our recent finding [34] demonstrating a role for PI 3-kinase α in *Listeria Monocytogenes* invasion may suggest a preventive effect of some flavonoids in the severe food-borne infections caused by this bacterial pathogen in humans.

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