

# Flavonoids as Aryl Hydrocarbon Receptor Agonists/Antagonists: Effects of Structure and Cell Context

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Chemoprotective phytochemicals exhibit multiple activities and interact with several cellular receptors, including the aryl hydrocarbon (Ah) receptor (AhR). In this study we investigated the AhR agonist/antagonist activities of the following flavonoids: chrysin, phloretin, kaempferol, galangin, naringenin, genistein, quercetin, myricetin, luteolin, baicalein, daidzein, apigenin, and diosmin. We also investigated the AhR-dependent activities of cantharidin and emodin (in herbal extracts) in Ah-responsive MCF-7 human breast cells, HepG2 human liver cancer cells, and mouse Hepa-1 cells transiently or stably transfected with plasmids expressing a luciferase reporter gene linked to multiple copies of a consensus dioxin-responsive element. The AhR agonist activities of the compounds (1 and 10  $\mu$ M) were as high as 25% of the maximal response induced by 5 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and their potencies were dependent on cell context. Galangin, genistein, daidzein, and diosmin were active only in Hepa-1 cells, and cantharidin induced activity only in human HepG2 and MCF-7 cells. Western blot analysis confirmed that baicalein and emodin also induced CYP1A1 protein in the human cancer cell lines. The AhR antagonist activities of four compounds inactive as agonists in MCF-7 and HepG2 cells (kaempferol, quercetin, myricetin, and luteolin) were also investigated. Luteolin was an AhR antagonist in both cell lines, and the inhibitory effects of the other compound were dependent on cell context. These data suggest that dietary phytochemicals exhibit substantial cell context-dependent AhR agonist as well as antagonist activities. Moreover, because phytochemicals and other AhR-active compounds in food are present in the diet at relatively high concentrations, risk assessment of dietary toxic equivalents of TCDD and related compounds should also take into account AhR agonist/antagonist activities of phytochemicals. **Key words:** agonists, Ah receptor, antagonists, flavonoids, interactions, TCDD. *Environ Health Perspect* 111:1877–1882 (2003). doi:10.1289/ehp.6322 available via <http://dx.doi.org/> [Online 22 August 2003]

Halogenated aromatic (HA) industrial by-products such as the polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) have been identified as mixtures in the environment, in foods, and in fish, wildlife, and human tissues (Safe 1990). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic HA compound and has been used as a reference standard for hazard and risk assessment of these environmental and dietary contaminants (Ahlborg et al. 1992, 1994; Birnbaum and DeVito 1995; Safe 1990, 1994; Van den Berg et al. 1998). The toxic equivalency factor (TEF) approach is being used for risk assessment of HA mixtures where the overall TCDD or toxic equivalents (TEQs) for the mixture is the sum of the concentrations of the individual congeners times their TEF value. The TEF approach for risk assessment of HA compounds is mechanism based because the compounds of concern all act through a common aryl hydrocarbon (Ah) receptor (AhR) and induce a common set of AhR-mediated responses. The TEF/TEQ concept is based on several assumptions that include persistence of the HA compounds, common mechanism of action, and response additivity for congeners in a mixture (Ahlborg et al. 1992, 1994; Birnbaum and DeVito

1995; Safe 1990, 1994; Van den Berg et al. 1998). There is good support for the validity of TEFs/TEQs for hazard and risk assessment of PCDDs and PCDFs. However, in mixtures containing PCBs, there is also evidence that for some AhR-mediated responses, nonadditive antagonist interactions can be observed (Safe 1998a, 1998b). For example, the antagonistic interactions between many environmentally significant PCBs, including 2,2',4,4',5,5'-hexachlorobiphenyl (PCB congener 153) interactions with TCDD or 3,3',4,4',5-pentachlorobiphenyl (PCB 126), for several AhR-mediated responses in several *in vivo* and *in vitro* models have been reported (Biegel et al. 1989; Davis and Safe 1988, 1989; Morrissey et al. 1992; Tysklind et al. 1995; Zhao et al. 1997a, 1997b). These results are consistent with a receptor-mediated pathway where both agonist and antagonist ligands are routinely identified. However, these results indicate that, among environmentally important HAs, additivity may not be observed for some responses, and this contradicts one of the key assumptions of the TEF/TEQ approach.

TEFs/TEQs have been extensively used for assessing potential dietary TEQ intakes from various foods, and regulatory agencies have used these data to develop guidelines for TEF/TEQ intake. For example, the World

Health Organization recently revised their tolerable daily intake value for TEQs from 10 pg/kg/day to 1–4 pg/kg/day (van Leeuwen et al. 2000). These guidelines also assume that TEQs are additive but do not address the increasing evidence that the AhR binds a host of endogenous chemicals, such as bilirubin, biliverdin, 7-ketocholesterol, and structurally diverse phytochemicals (Ashida et al. 2000; Bjeldanes et al. 1991; Casper et al. 1999; Chen et al. 1996; Chun et al. 2001; Ciolino et al. 1998a, 1998b, 1999; Ciolino and Yeh 1999; Denison et al. 1998; Gasiewicz et al. 1996; Gradelet et al. 1997; Phelan et al. 1998; Quadri et al. 2000; Savouret et al. 2001; Shertzer et al. 1999; Sinal and Bend 1997; Wang et al. 2001). Many of these phytochemicals, such as flavonoids, resveratrol, carotenoids, indole-3-carbinol, and related compounds, are weak AhR agonists/partial antagonists and are considered to be chemoprotective. This study further investigates a series of phytochemicals and their AhR agonist/antagonist activities; the compounds include the flavonoids chrysin, phloretin, kaempferol, galangin, naringenin, genistein, quercetin, myricetin, luteolin, baicalein, daidzein, apigenin, and diosmin, as well as cantharidin and emodin (in herbal extracts). Some of these compounds exhibit weak AhR agonist and antagonist activities in different cancer cell lines, and the results are interpreted in terms of their potential influence on the validity of the TEF/TEQ approach for risk assessment of HA compounds.

## Materials and Methods

**Chemicals, biochemicals, and cells.** The compounds used in this study were purchased from Sigma-Aldrich (Milwaukee, WI) and include chrysin (purity > 97%), phloretin (> 95%), kaempferol (> 95%), galangin (95%), naringenin (95%), genistein (98%), quercetin

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(99%), myricetin (95%), cantharidin (98%), luteolin (> 90%), baicalein (98%), daidzein (> 95%), emodin (> 90%), apigenin (> 90%), and diosmin (95%). These compounds were used without further purification. All compounds were dissolved in dimethyl sulfoxide (DMSO;  $10^{-2}$  M). Human MCF-7 breast cancer cells and HepG2 liver cancer cells were purchased from the American Type Culture Collection (Manassas, VA). M. Denison (University of California, Davis, CA) kindly provided the mouse Hepa-1 cells stably transfected with a dioxin-responsive element (DRE) promoter derived from the *CYP1A1* gene (Garrison et al. 1996). The transient transfection studies used a pDRE<sub>3</sub> construct, which contained three tandem consensus DREs (TCT TCT CAC GCA ACT CCG A—a single DRE sequence). The modified pGL2 vector contains a minimal TATA sequence between *Bgl*II and *Hind*III. We synthesized TCDD (purity > 98%) in this laboratory.

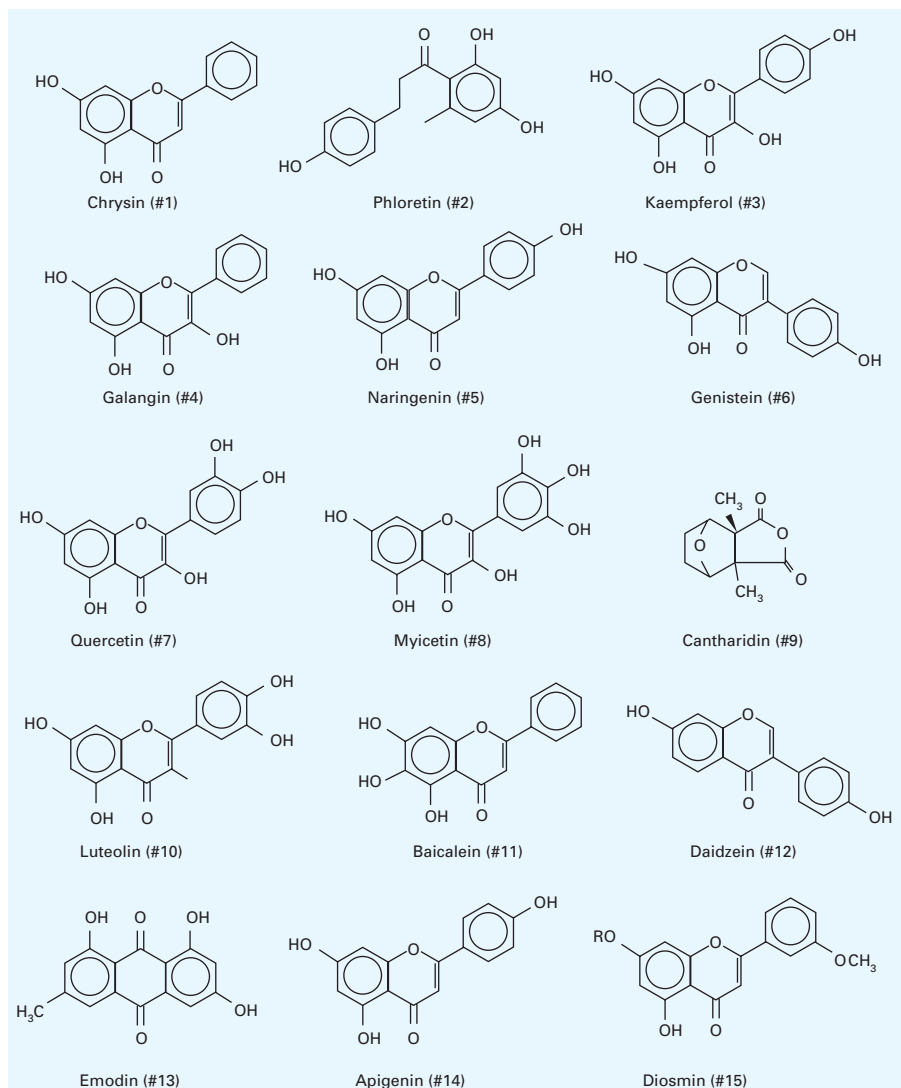
**DRE-dependent activation by 5 nM TCDD, flavonoids, cantharidin, and emodin.** Human MCF-7 cells, HepG2 cells, and stably transfected mouse Hepa-1 cells were maintained in Dulbecco modified Eagle medium (DME) supplemented with 5% fetal bovine serum (FBS), 2.2 g/L sodium bicarbonate, and 10 mL/L antibiotic/antimycotic solution. Cells for transient transfection assays were seeded in DME-F12 medium without phenol red and supplemented with 5% dextran-charcoal-stripped FBS, 2.2 g/L sodium bicarbonate, and 10 mL/L antibiotic/antimycotic solution. One day after seeding in DME-F12 and 5% stripped FBS, 1.5  $\mu$ g pDRE<sub>3</sub> was transfected into MCF-7 or HepG2 cells by calcium phosphate precipitation. Cells were also cotransfected with pCDNA3.1  $\beta$ -galactosidase ( $\beta$ -gal; 250 ng) (Invitrogen, Carlsbad, CA), which served as a control for transfection efficiency. Sixteen hours after transfection, media were removed, and fresh media containing the appropriate chemicals were added. Cells were grown for an additional 24 hr before harvesting with 200  $\mu$ L/well of reporter lysis buffer. Lysates were centrifuged at 40,000  $\times$  g, and luciferase and  $\beta$ -gal activities were determined with 30  $\mu$ L of the supernatant. Luciferase activity was determined using the luciferase assay system with reporter lysis buffer from Promega Corp. (Madison, WI).  $\beta$ -Gal activity was determined using the luminescent Galaction-Plus assay system from Tropix (Bedford, MA). The intensity of light emission from assays of cell extracts was determined using a lumicount luminometer (Perkin-Elmer, Boston, MA). Luciferase activity was normalized to  $\beta$ -gal activity for each treatment. Results are expressed as mean  $\pm$  SE for at least three determinations for each treatment group, and the fold induction (over DMSO) is shown in the figures.

**Western blot analysis.** We extracted whole-cell lysates using 1 $\times$  Western sampling buffer. Protein samples were heated at 100°C for 5 min, separated on 8% SDS-PAGE, and transferred to polyvinylidene difluoride (PVDF) membrane (Amersham, Piscataway, NJ). The PVDF membrane was blocked for 30 min and incubated with 1:1,000 CYP1A1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr at room temperature or with 1:1,000 AhR (Santa Cruz Biotechnology) overnight at 4°C. After vigorous washing for 20 min, 1:3,000 secondary antibody (Santa Cruz Biotechnology) was added, and the membrane was incubated with shaking for 45 min. After washing for 20 min, the membrane was incubated with ECL chemiluminescent substrate (NEN Life Science Products, Inc., Boston, MA) for 1 min, and exposed to Kodak X-Omat AR autoradiography film (Kodak, Rochester, NY). The membrane was reused and probed with the other antibody as indicated.

**Statistics.** All quantitative data were analyzed by analysis of variance followed by Fisher's protected least-significant-difference test for significance ( $p < 0.05$ ). Data from the transfection studies are expressed as mean  $\pm$  SE ( $n \geq 3$ ) for each treatment group.

## Results

AhR-mediated induction of CYP1A1 is a sensitive measure of Ah responsiveness. However, many phytochemicals interact with and inhibit CYP1A1 protein catalytic activity (Chen et al. 1996; Shertzer et al. 1999). Therefore, in this study we used a highly sensitive AhR-responsive assay (Denison et al. 1998) in which ligands activate the bacterial luciferase reporter gene activity in cells transfected with constructs containing multiple DRE promoter elements. Figure 1 illustrates structures of the 15 compounds used in this study; these include 12 flavonoids with different hydroxyl substitution patterns, plus the chemicals phloretin (a dihydrochalcone), cantharidin (a lactone), and



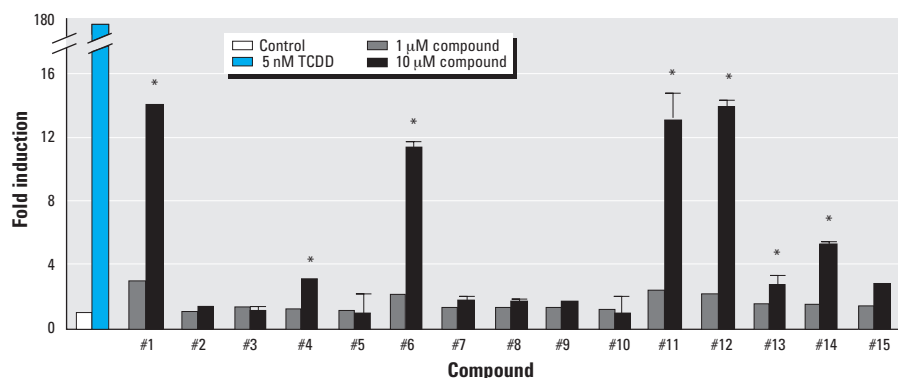
**Figure 1.** Structures of compounds used in this study.

emodin (an herbal laxative). Based on results of preliminary studies, we used 5 nM TCDD as a standard that induced maximal luciferase activity in stably transfected Hepa-1 cells (Figure 2) or in transiently transfected MCF-7 (Figure 3)

or HepG2 cells (Figure 4). Results from the stably transfected Hepa-1 cells demonstrate their sensitivity to 5 nM TCDD, with a 124-fold inducibility, whereas lower but significant induction was observed for chrysin,

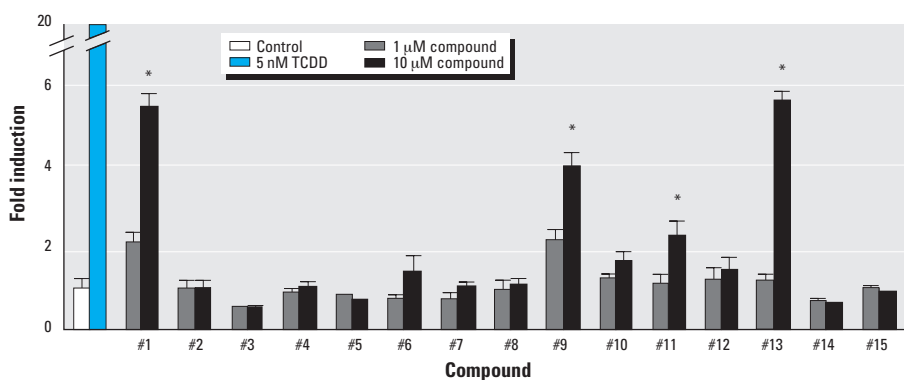
galangin, genistein, baicalein, daidzein, emodin, apigenin, and diosmin. Previous studies have also reported that emodin induced AhR-dependent CYP1A1 in human lung adenocarcinoma CL5 cells (Wang et al. 2001), and diosmin was also an AhR agonist in MCF-7 cells (Ciolino et al. 1998b). In contrast, the reported AhR agonist activity of quercetin in MCF-7 cells (Ciolino et al. 1999) was not observed in stably transfected Hepa-1 cells (Figure 2). Galangin exhibited AhR antagonist activity in BU-11, a murine B cell line (Quadri et al. 2000), but AhR agonist activity was observed in stably transfected Hepa-1 cells (Figure 2), and agonist activity of 60  $\mu$ M galangin has also been observed in Hepa-1 cells (Wang et al. 2001).

We further investigated the role of cell context in activation of transiently transfected pDRE<sub>3</sub> in human MCF-7 and HepG2 cell lines. At concentrations of 1 or 10  $\mu$ M, only chrysin, cantharidin, baicalein, and emodin activated luciferase activity in MCF-7 cells (Figure 3). With the exception of cantharidin, these compounds were also AhR agonists in stably transfected Hepa-1 cells, and compounds such as galangin, genistein, daidzein, apigenin, and diosmin that were active in Hepa-1 cells did not induce a response in MCF-7 cells. The pattern of induction responses in HepG2 cells was similar to that observed in MCF-7 cells in that chrysin, cantharidin, and baicalein activated gene expression, whereas (10  $\mu$ M) emodin was not active in this cell line (Figure 4). These data demonstrate that the AhR agonist activities of structurally diverse phytochemicals and cantharidin, which is derived from insect extract, are highly variable among different cell lines, and that their fold inducibility compared with TCDD is also dependent on cell context. The stably transfected Hepa-1 cells are more highly sensitive to the induction of luciferase activity by



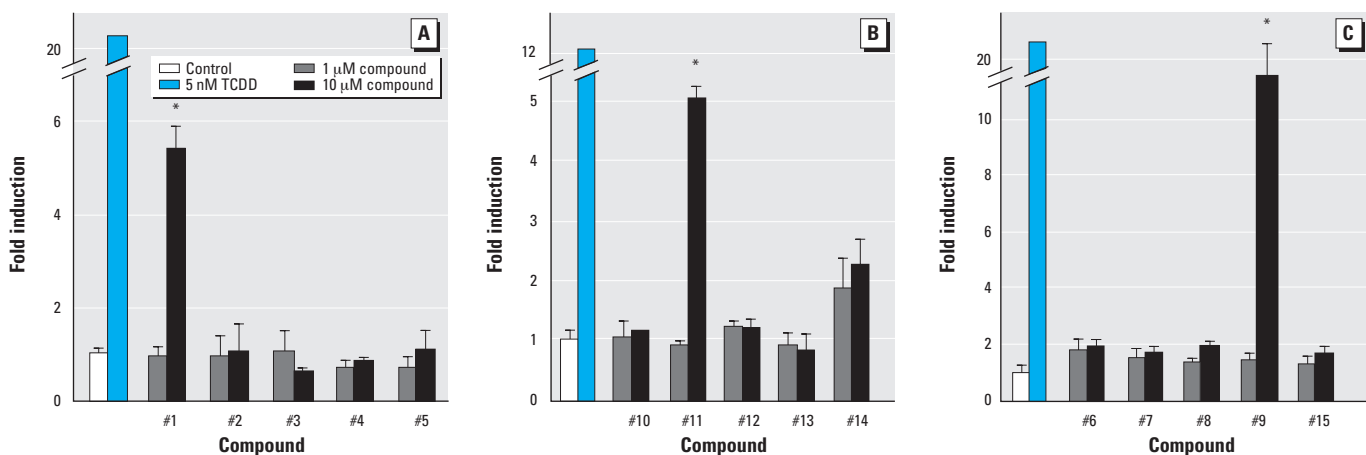
**Figure 2.** AhR-mediated transactivation in stably transfected Hepa-1 cells. Cells were treated with DMSO (control), 5 nM TCDD, or 1 or 10  $\mu$ M of the flavonoids for 24 hr; luciferase activity was determined as described in "Materials and Methods." Results are expressed as mean  $\pm$  SE for three replicate determinations for each treatment group. Compounds #1 through #15 are defined in Figure 1.

\*Significant induction,  $p < 0.05$ .



**Figure 3.** AhR-mediated transactivation in MCF-7 cells. Cells were transfected with pDRE<sub>3</sub> and treated with DMSO (control), 5 nM TCDD, or 1 or 10  $\mu$ M of the flavonoids, and luciferase activity was determined as described in "Materials and Methods." Results are expressed as mean  $\pm$  SE for each treatment group (three replicate determinations). Compounds #1 through #15 are defined in Figure 1.

\*Significant induction,  $p < 0.05$ .



**Figure 4.** AhR-mediated transactivation in HepG2 cells. (A) Compounds #1 through #5. (B) Compounds #10 through #14. (C) Compounds #6 through #9 and #15. Cells were transfected with pDRE<sub>3</sub> and treated with DMSO (control), 5 nM TCDD, or 1 or 10  $\mu$ M of the flavonoids, and luciferase activity was determined as described in "Materials and Methods." Results are expressed as mean  $\pm$  SE for three separate determinations for each treatment group. Compounds #1 through #15 are defined in Figure 1.

\*Significant induction,  $p < 0.05$ .

TCDD (5 nM) than to the other compounds. TCDD at 5 nM induced a 124-fold increase in luciferase activity, whereas only a 14-fold induction response was observed for 10  $\mu$ M chrysin. In contrast, 5 nM TCDD and 10  $\mu$ M chrysin, respectively, induced a 20- and 5.5-fold increase in luciferase activity in MCF-7 cells (Figure 3), and the potency of chrysin relative to TCDD was clearly higher in MCF-7 and HepG2 cells compared with stably transfected Hepa-1 cells.

The four compounds that activated luciferase activity in MCF-7 and HepG2 cells (chrysin, cantharidin, baicalein, and emodin) were also investigated as inducers of CYP1A1 protein in these cell lines (Figure 5). The highest nontoxic concentrations of each compound were used in the CYP1A1 protein induction assay because of the decreased sensitivity of this response compared with activation of luciferase activity in the transfected cells. With the exception of cantharidin, higher concentrations could be used because of the short duration (6 hr) of the experiment. Both baicalein and emodin increased CYP1A1 protein at concentrations of 100  $\mu$ M (MCF-7) or 50  $\mu$ M (HepG2), whereas chrysin was inactive at the same concentrations (Figure 5). In

the nontransfected cells, cantharidin exhibited high cytotoxicity, and CYP1A1 protein was induced only in MCF-7 cells (Figure 5B). In MCF-7 or HepG2 cells treated with 5 nM TCDD, there was a decrease in AhR protein levels as previously reported (Davarinis and Pollenz 1999; Ma and Baldwin 2000; Roberts and Whitelaw 1999; Wormke et al. 2000). In contrast, treatment with baicalein and cantharidin increased levels of the AhR protein, whereas no effects were observed after treatment with emodin or chrysin (Figure 5).

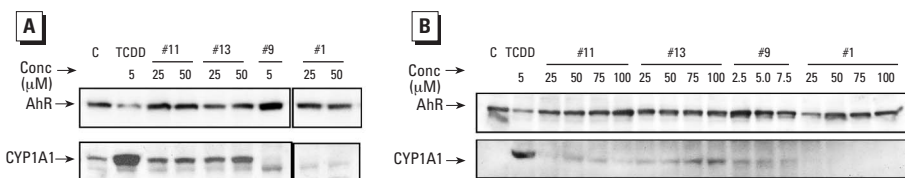
We also investigated the AhR antagonist activities of four compounds that were inactive in all three cell lines: kaempferol, quercetin, myricetin, and luteolin. Previous studies showed that quercetin was an AhR agonist and kaempferol was an AhR antagonist for induction of AhR-mediated CYP1A1 and DRE-dependent reporter gene activity in MCF-7 cells (Ciolino et al. 1999). However, in this study, cotreatment of MCF-7 cells with kaempferol or quercetin plus 5 nM TCDD resulted in significant inhibition of TCDD-induced luciferase activity at both concentrations (1 and 10  $\mu$ M) of flavone (Figure 6A). Myricetin (10  $\mu$ M) slightly decreased activity, whereas luteolin was a potent AhR antagonist.

In contrast, 1 or 10  $\mu$ M quercetin, kaempferol, and myricetin did not affect induction of luciferase activity by TCDD, whereas luteolin was an AhR antagonist in HepG2 cells (Figure 6B, C). These results demonstrate that AhR antagonist activities of these phytochemicals are also dependent on cell context.

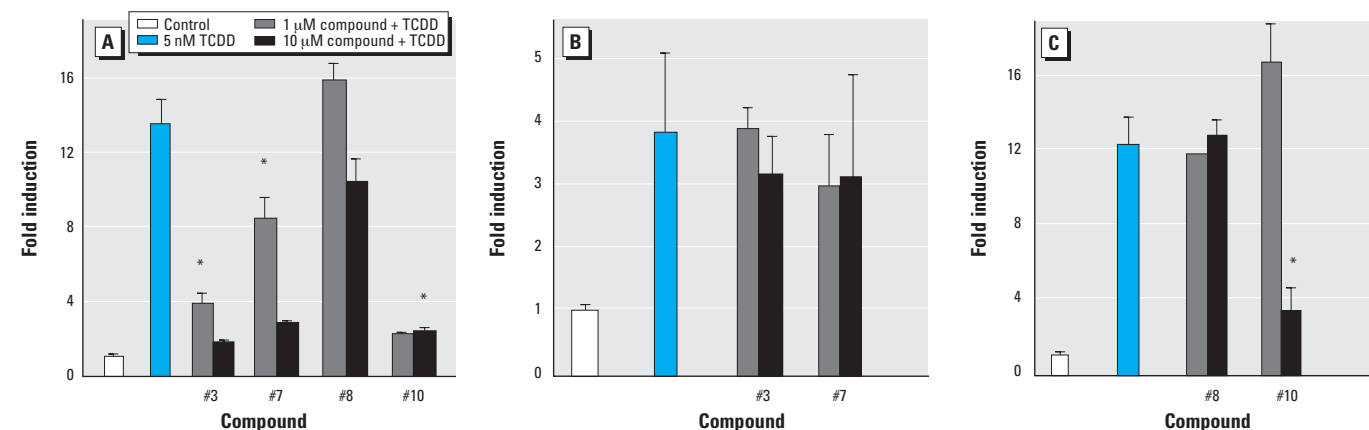
## Discussion

Results of this study demonstrate that several structurally diverse phytochemicals and cantharidin activate DRE-dependent luciferase (reporter gene) activity in cancer cell lines derived from mouse and human liver and human breast tumors. There are both similarities and differences in the AhR agonist activities of these compounds that are dependent on both structure and cell context. Our results show that TCDD, chrysin, and baicalein induced luciferase activity in all three cell lines. Cantharidin induced luciferase activity only in the human cells (MCF-7 cells, HepG2 cells), emodin was active in Hepa-1 and MCF-7 cells, and galangin, genistein, daidzein, apigenin, and diosmin were active only in stably transfected Hepa-1 cells. Previous studies have demonstrated that many of these compounds exhibit weak AhR agonist and/or partial antagonist activities in transactivation or receptor transformation assays (Ashida et al. 2000; Chun et al. 2001; Ciolino et al. 1998b, 1999; Quadri et al. 2000). However, it is apparent that there were some differences between this and other studies on the AhR agonist or antagonist activities of individual phytochemicals. For example, Ciolino et al. (1999) reported that quercetin and kaempferol exhibited AhR agonist and antagonist activities, respectively, in MCF-7 cells, whereas these compounds exhibited minimal AhR agonist activity in our studies in the same cell line (Figure 3).

There could be several explanations for differences in Ah responsiveness of phytochemicals



**Figure 5.** CYP1A1 protein induction by chrysin (#1), cantharidin (#9), baicalein (#11), and emodin (#13) in (A) HepG2 cells or (B) MCF-7 cells. Abbreviations: C, control; Conc, concentration. Cells were treated with DMSO (control), 5 nM TCDD, or different concentrations of the flavonoids for 6 hr. Whole-cell lysates were then prepared, and CYP1A1 and AhR proteins were detected by Western blot analysis as described in "Materials and Methods." These experiments were determined at least two times for each cell line. Comparable results were obtained showing increased CYP1A1 protein after treatment with baicalein (#11; MCF-7/HepG2), emodin (#13; MCF-7/HepG2), and cantharidin (#9; MCF-7). CYP1A1 protein induction was not observed for chrysin (#1).



**Figure 6.** AhR antagonist activities of phytochemicals in MCF-7 and HepG2 cells. (A) MCF-7 cells treated with kaempferol (#3), quercetin (#7), myricetin (#8), or luteolin (#10). (B) HepG2 cells treated with kaempferol (#3) or quercetin (#7). (C) HepG2 cells treated with myricetin (#8) and luteolin (#10). Cells were transfected with pDRE<sub>3</sub> and treated with DMSO (control) or with 5 nM TCDD alone or in combination with 1 or 10  $\mu$ M concentrations of the compounds, and luciferase activities were determined as described in "Materials and Methods." Results are expressed as mean  $\pm$  SE for three separate determinations for each treatment group.

\*Significant inhibition,  $p < 0.05$ .

in the Hepa-1, MCF-7, and HepG2 cells. The stably transfected mouse Hepa-1 cell line was more sensitive than the transiently transfected human MCF-7 and HepG2 cells to TCDD and to most of the phytochemicals. This could be due to the stable integration of the construct and the presence of four DREs compared with three DREs in the transiently transfected pDRE<sub>3</sub> used in the HepG2 and MCF-7 cell studies (Figures 3 and 4). In addition, the mouse AhR expressed in Hepa-1 cells exhibits higher binding affinity for TCDD than does the human AhR (Ema et al. 1994), and structural differences in the mouse and human AhR may also affect the binding and transactivation activities of the phytochemicals. Chrysin (10 μM) was the most consistent inducer in the reporter gene assays in the three cell lines (Figures 2–4). However, at concentrations as high as 100 and 50 μM in MCF-7 and HepG2 cells, respectively, induction of CYP1A1 protein was not observed (Figure 5). This illustrates the high sensitivity of the reporter gene assays for detecting AhR agonists and suggests that relative compound potencies in this assay may be different for other AhR-mediated responses (Figure 5). This has been observed for TCDD and related compounds that also exhibit species- and response-specific potency differences (Safe 1990). Like the nuclear hormone receptors, ligand-induced activation of the AhR is dependent on interactions with nuclear coregulatory proteins (Beischlag et al. 2002; Kumar et al. 1999; Nguyen et al. 1999). Nevertheless, results of this and other studies clearly demonstrate that structurally diverse phytochemicals exhibit AhR agonist activities.

We have also investigated interactions of kaempferol, quercetin, myricetin, and luteolin as AhR antagonists in MCF-7 and HepG2 cells (Figure 6) because these compounds alone at concentrations of 1 or 10 μM did not induce luciferase activity in these cell lines (Figures 3 and 4). The results showed that luteolin blocked TCDD-induced luciferase activity in both cell lines, and these results were comparable with the inhibition of TCDD-induced transformation of the rodent cytosolic AhR as previously reported (Ashida et al. 2000; Thenot et al. 1999). The AhR antagonist activities of kaempferol, quercetin, and myricetin were dependent on the cell context (Figure 6). Myricetin exhibited weak (but not significant) antagonist activity only in MCF-7 cells, and both kaempferol and quercetin were also antagonists in MCF-7 but not HepG2 cells. Because many flavonoids activate the estrogen receptor (ER), it is possible that inhibitory ER–AhR crosstalk that has previously been reported (Jeong and Lee 1998; Ricci et al. 1999) may contribute to AhR antagonist activities observed in MCF-7 cells (Figure 6). It is possible that higher concentrations of compounds

1–15 (Figure 1) may exhibit AhR agonist/antagonist activities. However, higher concentrations were not investigated because of cytotoxicity.

Several studies show that phytochemicals weakly activate the AhR in one or more assays and also act as AhR antagonists. These compounds include kaempferol (Ciolino et al. 1999), resveratrol (Casper et al. 1999; Ciolino and Yeh 1999), galangin (Quadri et al. 2000), rhapontigenin (Chun et al. 2001), indole-3-carbinol (Chen et al. 1996), and diindolylmethane (Chen et al. 1996). Ashida et al. (2000) also showed that ≤ 25 μM concentrations of various phytochemicals block TCDD-induced transformation of rat liver cytosolic AhR, and these include chrysin, baicalein, apigenin, luteolin, tangeretin, galangin, kaempferol, fisetin, morin, quercetin, myricetin, tamarixetin, isorhamnetin, naringenin, eriodictyol, and hesperitin. Total daily intakes of dietary flavonoids may be as high as 1 g (Verdeal and Ryan 1979), and serum levels of some flavonoids such as quercetin and genistein can be in the nanomolar to low micromolar range. The overall serum concentrations of most phytochemicals in humans is unknown. However, levels are probably in the nanomolar to micromolar range and are dependent on the food product and clearance times for individual compounds. 7-Ketocholesterol is also an AhR antagonist with a competitive binding IC<sub>50</sub> value (concentration that inhibits 50%) of 500 nM (Savouret et al. 2001), and plasma concentrations of this compound range from 20 to 200 nM in healthy humans (Dzeletovic et al. 1995). This would suggest that many phytochemicals and endogenous compounds with AhR agonist/antagonist activities are present in human serum.

Risk assessment of HA compounds uses the TEF/TEQ approach. For example, daily TEQ intakes of TCDD and related compounds are 50–200 pg in most countries, and these values have substantially decreased over the past 10 years (van Leeuwen et al. 2000). Serum TEQ values are < 5 ppt (lipid weight) or approximately 0.1 pM for TCDD and related compounds, whereas serum levels of some “natural” AhR agonists are in the nanomolar to low micromolar range. Thus, the serum ratios of flavonoids/TCDD TEQs are 10<sup>4</sup> to 10<sup>6</sup>, and these ratios are similar to those required for inhibition of TCDD-induced responses by some phytochemicals (Ashida et al. 2000; Chun et al. 2001; Ciolino et al. 1998b, 1999; Quadri et al. 2000). Results shown in Figure 5 demonstrate that 1 μM luteolin inhibited (> 90%) TCDD-induced transactivation in MCF-7 cells at flavonoid/TCDD ratios as low as 200/1. Moreover, ratios of PCB 153/TCDD TEQs in human tissues are also > 10<sup>4</sup>, which is comparable

with ratios required for PCB 153–mediated inhibition of several TCDD-induced biochemical and toxic responses (Safe 1998a, 1998b). It is likely that dietary intakes of most phytochemicals would be below levels required for an AhR agonist response based on results from cell culture studies. The potential chemoprotective effects of the expanding list of AhR-active phytochemicals and related compounds on TCDD-TEQ–mediated adverse responses should be further investigated in *in vivo* models. These results can then be used for development of recommended dietary TCDD-TEQ values that reflect the combined intake of HA compounds plus high levels of “natural/phytochemical” AhR antagonists/agonists.

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