Review

Influence of food polyphenols on aryl hydrocarbon receptor-signaling pathway estimated by *in vitro* bioassay

Yoshiaki Amakura a,b,*, Tomoaki Tsutsumi b, Kumiko Sasaki b, Masafumi Nakamura c, Takashi Yoshida a, Tamio Maitani b

a College of Pharmaceutical Sciences, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578, Japan
b Division of Foods, National Institute of Health Sciences, 1-18-1 Kamiyaga, Setagaya-ku, Tokyo 158-8301, Japan
c Hiyoshi Corporation, 908 Kitaosaka-cho, Omihamamachi, Shiga 523-8555, Japan

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Abstract

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxic and biological actions of many aromatic environmental pollutants such as dioxins. We investigated AhR activation by some vegetable constituents, including flavonoids, tannins, and related polyphenols, using an AhR-based *in vitro* bioassay for dioxins. Among the compounds tested, marked AhR activation was exhibited by isoflavones such as daidzein, resveratrol (a stilbene) structure, some flavonones such as naringenin, and flavones such as baicalein. On the other hand, some flavones such as apigenin, flavonols such as quercetin, and anthraquinones such as emodin, showed notable inhibitory effects on the *in vitro* activation of AhR induced by the dioxin [2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)]. In addition, AhR-mediated interactions between AhR and some plant extracts, including those from vegetables, fruits, herbs, and teas, were tested by using the AhR-based bioassay. Of the samples tested, some leafy green vegetables, citrus fruits, and herbs that contain food polyphenolics showed AhR-based interactions at high concentrations. On the basis of these finding, we discuss the implications of polyphenolics on the AhR-signaling pathway.

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Keywords: Polyphenol; Aryl hydrocarbon receptor; Vegetable food; *In vitro* bioassay; Dioxin

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* Corresponding author. Address: College of Pharmaceutical Sciences, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578, Japan. Tel.: +81 89 925 7111; fax: +81 89 925 7162.
E-mail address: amakura@cc.matsuyama-u.ac.jp (Y. Amakura).

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1. Introduction

The aryl hydrocarbon receptor (AhR), which is also referred to as a dioxin receptor, is a basic helix-loop-helix (bHLH)- and Per-Arnt-Sim (PAS)-containing transcription factor. It is present in numerous animal species, including humans and tissues, and activates gene expression in a ligand-dependent manner (Schmidt and Bradfield, 1996; Ma, 2001; Denison et al., 2002; Mimura and Fujii-Kuriyama, 2003) (Fig. 1a). The prototype ligand is known as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), an archetypical dioxin known as one of the most potent congeners. Other known ligands are environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) and persistent organochlorine pollutants (POPs), including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (Safe, 1986, 1990; Whitlock, 1993; Denison et al., 2002). Unliganded AhR is present in the cytosol of most cells, forming a complex with a dimer of 90-kDa heat shock protein (Hsp90), an α-associated protein 2 (XAP2), and a 23-kDa co-chaperone protein (p23). Ligand binding to AhR leads to nuclear translocation, followed by release of its associated protein subunits (Hsp90, XAP2, and p23) and heterodimerization with the AhR nuclear translocator protein (Arnt). This AhR/Arnt heterodimer binds to DNA sequences, called xenobiotic responsive elements (XRE), which are distributed in the enhancer regions of dioxin-responsive genes, and regulate the expression of target genes including drug-metabolizing enzymes, such as cytochrome P450 (CYP) 1A1. Accordingly, this DNA interaction is highly correlated with the initial step of subsequent toxicity events including carcinogenicity, developmental and reproductive toxicity, and immunological impairment that are known as dioxin toxicity effects (Landers and Bunce, 1991; Poellinger, 2000; Denison and Nagy, 2003; Mimura and Fujii-Kuriyama, 2003; Mandal, 2005; Schwarz and Appel, 2005) (Fig. 1b).

The lack of TCDD toxicity in AhR knockout mice, along with the ability of AhR to act as a ligand-dependent transcription factor, indicated that AhR mediates the toxic and biological effects of TCDD (Mimura et al., 1997). Recently, research on the structure and physiological functions of AhR cell cycle regulation has been reported (Bock and Köhle, 2006; Harper et al., 2006; Pandini et al., 2007; Goryo et al., 2007), and the characterization of AhR has gradually been clarified. However, AhR is still relatively poorly understood, because its physiological ligand, mechanisms, and functions remain largely unknown. The present functional role of AhR was derived mainly based on studies using environmental contaminants such as dioxins. Environmental contaminants that are prototype AhR ligands are artificial products that have appeared recently. Therefore, AhR might primarily function in human health.

![Diagram](https://example.com/diagram.png)

**Fig. 1.** (a) Domain structure of the aryl hydrocarbon receptor (AhR). bHLH, basic helix-loop-helix; PAS, Per-Arnt-Sim domain; Q-rich, glutamine rich region; Arnt, AhR nuclear translocator protein; XRE, xenobiotic-response element. The PAS domain contains two structural repeats (PAS A and PAS B). (b) Mechanistic model of the AhR signaling pathway. See text for detailed description.
as a regulatory receptor for exogenous natural products such as food constituents.

To better understand AhR's inherent physiological role and significance, information on the interactions between it and compounds such as food constituents related to people's lives is needed, and more fundamental data is required. Recently, numerous investigations have been carried out to search for the agonists and antagonists of AhR contained in natural products rather than in environmental contaminants (Denison et al., 2002; Denison and Nagy, 2003). Regarding AhR agonists, AhR transformation has reportedly been induced by the following: tryptophan and its metabolites (Heath-Pagliuso et al., 1998), carotenoids (β-apo-8'-carotenal, canthaxanthin, and astaxanthin) (Gradelet et al., 1997), berberine (Vrzal et al., 2005), indole-3-carbinol (Bjeldanes et al., 1991; Chen et al., 1996), bilirubin, biliverdin (Phelan et al., 1998), flavonoids (Ashida et al., 2000), and others. Recently, tunicamycin, a well-known antibiotic, has been identified as an agonist of the AhR-XRE signal pathway (Horikawa et al., 2006). Indigo and indirubin were also shown to be endogenous AhR agonists present in human urine (Adachi et al., 2001; Sugihara et al., 2004). The AhR agonist activity of the extracts of dietary herbal supplements, vegetables, and fruits were also assessed (Jeukend et al., 2003). AhR agonists are noted as physiological regulatory factors, while the toxicity of a dioxin-like compound is doubtful.

The following have been reported to function as AhR antagonists that involve inhibition of the AhR-signaling pathway: flavonoids (apigenin, luteolin, kaempferol, quercetin, galangin, etc.) (Reiners et al., 1999; Ciolino et al., 1999; Ciolino and Yeh, 1999; Ashida et al., 2000; Fukuda et al., 2004; Ishida et al., 2005; Hamada et al., 2006), catechins (Williams et al., 2000; Ashida et al., 2000; Fukuda et al., 2004), curcumin (Ciolino et al., 1998a), resveratrol (Ciolino et al., 1998b; Casper et al., 1999), and lutein (Fukuda et al., 2004). Recently, the suppressive effects of anthocyanidins and/or anthocyanins and extracts of black tea, molokhia, and propolis on the dioxin-induced transcription of AhR have also been investigated (Park et al., 2004, 2005; Fukuda et al., 2005; Mukai et al., 2005; Nishiumi et al., 2006). These studies suggest that AhR antagonists might protect against dioxin toxicity.

This review summarizes our recent investigations, which include the interaction between polyphenol constituents and AhR as determined by in vitro bioassay. The influence of polyphenols on the AhR-signaling pathway with regard to human health are also discussed.

2. AhR activation by polyphenol constituents determined using in vitro bioassay

The in vitro AhR-inducing potencies of plant constituents, mainly polyphenol compounds, that are present in vegetables, fruits, teas, and herbs (Amakura et al., 2003a) were investigated. For identification of AhR-activating compounds, an in vitro reporter gene assay, called the chemical activated luciferase gene expression (CALUX) assay (Denison et al., 1998), was used. The mechanistic outline of the assay is depicted in Fig. 2a. This assay, which uses mouse hepatoma cells (Hepa ilec7) containing a stably transfected AhR-responsive luciferase reporter gene, detects dioxin-like compounds based on their ability to activate AhR. Since the response for a sample containing dioxin-like compounds can be correlated with dioxin levels in the CALUX assay, this assay has recently been applied as an alternative screening method to determine dioxin levels (Tsutsumi et al., 2003). With this assay, TCDD showed an appreciable, dose-dependent increase in luciferase activity (Fig. 3). The concentrations of a test compound producing luciferase activity equal to 25% and 50% of the maximal response to TCDD were calculated and expressed as EC50 and EC50, respectively. EC25 and EC50 of TCDD were determined to be 1.3 × 10⁻⁵ and 3.0 × 10⁻³ µM, respectively. Dose-response curves plotted

Fig. 2. Outline of mechanistic models of AhR-mediated in vitro bioassay used in this study. (a) CALUX assay, (b) AhR-immunoassay.
on a log scale for some samples are also shown in Fig. 3. Most tested compounds showed no luciferase induction at the concentration level of 100 μM, but some showed luciferase activity at higher concentrations. Of the samples tested, isoflavones produced responses that reached maximal TCDD levels (Fig. 3 and Table 1). EC₅₀(TCDD) values for daidzein (1), genistein (2), and genistein (3) were 3.0 (7.9), 4.2 (20.6), and 2.4 (7.0) μM, respectively. Their glycosides showed lower AhR responses than the corresponding aglycones (EC₅₀(TCDD): 12.0, 20.0, and 4.2 μM for daidzin (4), glycin (5), and genistin (6), respectively), and their acetates or malonylates showed much lower induction (EC₅₀(TCDD): 32.0, 48.0, and 98.0 μM for 6'-acetyldaidzin (7), 6'-malonyldaidzin (8), and 6'-malonylgenistin (9), respectively).

Among several flavonones, naringenin (10) and hesperetin (11) elicited agonist-like AhR-mediated activity (EC₅₀(TCDD): 5.3 and 38.0 μM). Their glycosides (naringin and hesperidin) and flavanones including (+)-taxifolin and (+)-fustin did not induce activity at the EC₅₀(TCDD) level. The tendency for glycosides to weaken these activities was similar to that of isoflavones. Of the flavones, baicalin (12) and baicalin (13) induced the production of luciferase activity (EC₅₀(TCDD): 2.8 and 3.2 μM). Chrysine (14) slightly induced the activity at 14.0 μM in EC₅₀(TCDD). On the other hand, apigenin (17), luteolin (18), and others slightly induced the activity at high concentrations in the order of 10–100 μM, but no luciferase induction reached the EC₅₀(TCDD) level. Also, flavonols, myricetin (21), morin (22), and others slightly activated luciferase at high concentration on the order of 100 μM.

Based on these findings, the structure–activity correlations in the activation of AhR by flavonoids suggested that the level of activity depended on the molecular size, polarity, and structure of isoflavones and flavonones. Isoflavones such as daidzein (1), genistein (2), and genistein (3) had similar AhR-inducing potencies, while their glycosides and 6'-O-acetylates showed lower induction levels. Similarly, flavanone glycosides had weaker activity than their corresponding aglycones such as naringenin (10) and hesperetin (11), indicating that the increase in the molecule's polarity clearly weakened the activity. Flavanone 3-ols and flavan 3-ols such as (+)-catechin and (−)-epicatechin showed poor induction of luciferase activity. Therefore,
Table 1

<table>
<thead>
<tr>
<th>Relative responses of the reporter gene system to some polyphenolic constituents (data from Amakura et al., 2003a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EC_{TCDD20} (µM)</strong></td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
</tr>
<tr>
<td>Isoflavones</td>
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<tr>
<td>Daidzein (1)</td>
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<tr>
<td>Glycitein (2)</td>
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<tr>
<td>Genistein (3)</td>
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<tr>
<td>Daidzin (4)</td>
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<tr>
<td>Glycitein (5)</td>
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<tr>
<td>Genistein (6)</td>
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<tr>
<td>6'-Acetyldaidzin (7)</td>
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<tr>
<td>6'-Malonyldaidzin (8)</td>
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<td>6'-Malonylgenistein (9)</td>
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<tr>
<td>Flavonones</td>
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<td>Naringenin (10)</td>
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<td>Hesperetin (11)</td>
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<td>Flavones</td>
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<td>Baicalein (12)</td>
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<td>Baicalin (13)</td>
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<tr>
<td>Chrysin (14)</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Resveratrol (15)</td>
</tr>
<tr>
<td>Alizarin (16)</td>
</tr>
</tbody>
</table>

Each value is the mean of at least three replicates.

* Concentration producing luciferase activity equal to 25% (or 50%) of the maximal response to TCDD. Calculated from the slope of the linear portion of each dose-response curve near the origin.

b No luciferase induction to the EC_{TCDD50} level observed.

Fig. 4. Structures of TCDD and some tested polyphenols.

Fig. 5. Molecular models of AhR-activating polyphenols obtained as MM2-minimized structures, (a) TCDD; (b) daidzein (1); (c) resveratrol (15); (d) alizarin (16); (e) baicalein (12). Energy-minimized structures were calculated by molecular mechanics using MM2 in CS Chem 3D.
the hydroxyl group at C-3 of the C-ring may also contribute to weakening the activity. Baicalein (12), baicalin (13), and chrysin (14), which induced AhR activation, each possess two or three hydroxyl groups at C-5, -6, and -7 of the A-ring, but none on the B-ring. Other flavones, apigenin (17), luteolin (18), and vitexin, which weakly induced the activity, each have a hydroxyl group on the B-ring. As for flavones, hydroxyl groups on the B-ring reduced the activation, while the hydroxyl groups on the A-ring had a negligible influence. Similarly, flavonols, which possess hydroxyl group(s) on the B-ring, poorly induced activation, with the exception of myricetin (21) and morin (22), each of which caused a slight induction at the 100 μM level. Thus, in naturally occurring flavones and flavonols, hydroxyl group(s) on the B-ring and in C-3 of the C-ring may reduce activation.

Among the other compounds tested, resveratrol (15), having a trans-stilbene structure, showed strong AhR-inducing potency comparable to the maximum induction of TCDD at a high concentration level (7.3 (34.3) μM in EC_{50} (EC_{90})). While analogues possessing longer carbon chains [rosmarinic acid, curcumin (35)] showed significantly lower AhR-inducing capabilities. Previously, the AhR ligand activity of trans-stilbene was reported, so the present result further demonstrated that the molecular size in the trans-stilbene is important for this activity (Kato et al., 2002). Among the anthraquinones, alizarin (16) showed AhR activation (30.0 μM in EC_{50}), whereas emodin (32) and aloe-emodin (33) exhibited only weak AhR activity. Although they each possess a structure similar to that of TCDD, substituents (–OH, –CH3, and –CH2OH) in emodin (32) and aloe-emodin (33) may contribute less than in alizarin (16) to the activity. Condensed and hydrolyzable tannins, phenolic carboxylic acids, rosmarinic acid, and curcumin (35) induced little production of luciferase at EC_{50} levels.

Fig. 5 depicts molecular models of TCDD and several compounds that induced luciferase activity [daidzein (1),

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Fig. 6. Dose-dependent inhibitory effect of some vegetable polyphenols on AhR-activation induced by TCDD in Ah-1. Each point represents the mean of two or three replicates.
resveratrol (15), alizarin (16), and baicalein (12); these
to achieve them for their minimum energy con-
formation. The AhR has been found to favor hydrophobic
molecules with quasi-planar structures and to accommo-
date a ligand-binding pocket (Safe, 1986; Landers and
Bunce, 1991; Denison and Nagy, 2003). As shown in
Fig. 5, these active compounds have molecular sizes and
planar structures similar to those of TCDD.
Isoflavones such as daidzein (1), the so-called phyto-
estrogens, exhibited AhR activation in this in vitro
experimental system. Southeast Asian and Japanese people
often consume soybeans and soybean-derived products,
which contain isoflavones in abundance. It is also known
that these soy isoflavones may have some health-enhancing
properties (Murkies et al., 1998; Setchell and Cassidy,
1999). Therefore, it is considered that a modest AhR- induc-
er including foods may perform some beneficial regulatory
role in the homeostasis, proliferation, and differentiation
of cells in animals (Harper et al., 2006).
Isoflavones are known to be mostly metabolized in the
body, and their metabolites may not induce AhR because
equil, which is regarded as the final metabolite of isoflav-
one, only slightly AhR activity at high concentra-
tions. On the other hand, it has also been reported that two
soy isoflavones, daidzein (1) and genistein (2), appear to be
incorporated into tissues after ingestion of baked soybean
powder by humans (Watanabe et al., 1998). Since the daily
intake and serum excretions of daidzein (1) and genistein
(2) by Japanese has been assessed [daily intake of daidzein
(1) and genistein (2) were ca. 20 and 30 mg/day, respec-
tively; serum excretions were ca. 120 and 475 nM, respec-
tively] (Yamamoto et al., 2001), it is clear that they are
incorporated intact into the body. Considering the bio-
availability of isoflavones in these reports, it can be inferred
that, in the quantities typically consumed, these isoflavones
might not function as agonists to AhR, while a large excess
intake of AhR-inducers such as isoflavones may merit
attention as a risk factor for health.

3. Inhibitory effects of polyphenol constituents on the AhR-
mediated activity induced by TCDD

To evaluate the effect of polyphenol constituents on the
AhR pathway induced by TCDD, we used an AhR-based
bioassay for dioxins, the Ah-Immunobay (Ah-I Paracel-
sian, USA), which previously proved sensitive as a prelimi-
nary experimental model (Amakura et al., 2003b). The
Ah-I kit method is a receptor-binding assay using cytosol
containing AhR extracted from mammalian liver cells. It
immunologically measures the dioxin level by utilizing an
antigen-antibody reaction (Fig. 2b). This technique detects
the reactivity of AhR with dioxins and dioxin-like com-
ounds on an ELISA plate without using living cells. It is
useful for screening the biological toxicity of dioxins
(Kobayashi et al., 2002).

Fig. 6 shows the dose–response curves plotted on a log
scale for some individual samples (see Figs. 4 and 7 for
structures). Most of the compounds inhibited AhR activa-
tion by TCDD at high concentrations around the 50 µM
level. Some showed marked inhibitory effects at low con-
centrations of 0.5–2.5 µM. The concentrations showing
AhR activity equal to 70% of the maximal response to
TCDD in controls were calculated and expressed as EC_{70}
values. Table 2 shows the EC_{70} values of the compounds
on AhR-based bioassay activation. The inhibitory effects

| Table 2 | Inhibitory effects of some polyphenolic constituents on TCDD-induced activation of AhR estimated using the AhR-based bioassay (data from Amakura et al. (2003b)) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Flavonoids** | **EC_{70} (µM)** | **Hydrolyzable Tannins** | **EC_{70} (µM)** |
| Apigenin (17)   | 1.9             | Pentagalloylgucose (25) | 29.6            |
| Luteolin (18)   | 1.8             | Pedunculagin (26)      | 42.0            |
| Baicalein (12)  | 5.1             | Tellimagrandin I (27)  | 12.4            |
| Chrysirin (14)  | 0.7             | Geranicin (28)         | 27.3            |
| **Flavonols**   |                 | Casuarin (29)          | 29.3            |
| Quercetin (19)  | 2.7             | Aegimone (30)          | 6.4             |
| Kaempferol (20) | 2.1             | Gemin A (31)           | 31.5            |
| Myricetin (21)  | 4.3             | Others                |                 |
| Morin (22)      | 5.3             | Emodin (32)            | 0.6             |
| Mirtocresol (33) | 2.1             | Aloemodin (33)         | 0.5             |
| Naringenin (16) | 27.7            | Alizarin (46)          | 3.2             |
| Hesperetin (11) | 14.6            | Brevifolin/carboxylic acid (34) | 3.9 |
| Resveratrol (15) | 3.9             | Curcumin (35)          | 35.4            |
| Catechin (36)   | 5.6             |                        |                 |

Each value is the mean of at least three replicates.

* Concentration producing AhR activity equal to 70% of the maximal response to TCDD. Calculated from the slope of the linear portion of each dose–
response curve near the origin.
of most samples were weak, less than the EC_{50} level even at high concentrations (inhibitory effect ca. 20%).

The flavones and flavonols in Table 2 had strong inhibitory potencies (EC_{50}: 0.7–3.3 μM) against AhR activation induced by TCDD. However, three flavonol 3-O-glycosides, quercitrin, rutin, and isoquercitrin, did not inhibit activity to the EC_{50} level. In contrast, spiraeoside (23) (a quercetin 4'-O-glucoside) showed a strong inhibitory effect, comparable to that of the aglycone [quercetin (19)]. This suggested that the inhibitory effects of flavonols might be little influenced by glycosidation of the B-ring. Among the flavanones, hesperetin (11) and naringenin (10) showed inhibitory effects with EC_{50} values of 14.6 and 27.7 μM, while their 7-O-glycosides (naringin, hesperidin, taxifolin, and fustin) did not inhibit activity to the same level. The tendency of glycosides to weaken these activities was similar to those of flavones and flavonols. The isoflavones did not show an inhibitory effect on AhR activation at the EC_{50} level, and they slightly inhibited activity at high concentrations of 25–50 μM (ca. 10–20% inhibition), although

![structures of some tested polyphenols](image-url)
genistein (3) had a slight inhibitory effect at 40.8 μM in 
EC70 and equal inhibited activity at 41.2 μM.

Anthraquinones showed remarkable inhibition of AhR 
activation, comparable to flavones and flavanols [EC70 
values of aloes-emedin (33), emodin (32), and alizarin 
(16) were 0.5, 0.6, and 3.2 μM, respectively]. Resveratrol 
(15), brevifolin-carboxylic acid (34), coumestrol (36), and 
curcumin (35) also inhibited activation in this assay 
(EC70 values were 3.9, 3.9, 5.6, and 35.4 μM, respectively). 
Among these, resveratrol (15) and curcumin (35) were 
reported to show antagonist effects on AhR (Ciolo et al., 
1998a, b; Casper et al., 1999). As they also showed 
inhibitory effects in our assay system, they would be 
promising candidates as prophyllactic agents for the 
preservation of dioxin toxicity.

On the other hand, some hydrolyzable tannins in Table 
2, which are large molecules with molecular weights of 
1000–2000 higher than the phenolics mentioned above, 
inhibited AhR activation with EC70 of 6.4–42.0 μM, while 
condensed tannins showed no inhibitory effects. As tannins 
are known to form complexes with various proteins, to 
function as enzyme inhibitors, and so on (Haslam, 1989), 
their inhibitory effects in this assay system may partly be 
ascribed to a non-specific binding to AhR. Further study 
will be required to elucidate these effects.

In Section 2, we described the AhR-mediated activity of 
vegetable polyphenolics using the CALUX assay. The 
active compounds were classified into the so-called phyto-
estrogens, and their structural characteristics were similar 
to those of TCDD. Flavones such as chrysin (14) 
showed remarkable activation in the AhR-based assay as 
well as strong inhibitory effects on AhR-mediated activity 
induced by TCDD at the EC70 level, suggesting that they 
may be both agonists and antagonists of AhR, depending 
on the amount. On the other hand, flavonoids such as apige-
enin (17) and flavonols were regarded as strong antagonists 
of AhR because they showed little AhR activation. Most 
isoflavones had slight inhibitory effects, less than the 
EC70 level, whereas genistein (3) and equol (24) showed 
inhibition at the EC70 level. Anthraquinones, brevifolin-
carboxylic acid (34), and coumestrol (36), each of which 
slightly activated AhR at high concentrations, also pro-
duced inhibitory effects on AhR activity at the EC70 level.

Some reports have measured the amounts of flavonoid 
ingestion and absorption in the human body. For example, 
the daily consumption of flavonoids was recently estimated 
at only 20–35 mg/day (Manach et al., 2005). Another paper 
reported that the ingestion of 8, 20, and 50 mg quercetin 
(19) resulted in concentrations of 0.14, 0.22, and 0.29 μM 
quercetin (19) in plasma, respectively (Yamamoto et al., 
2001). Another study, performed on ten healthy volunteers, 
showed that ingestion of 68 ± 13 mg quercetin equivalents 
from onion resulted in a maximum quercetin (19) 
concentration in plasma of 0.74 ± 0.15 μM (Holman et al., 
1997). Taking into account the average intake (ca. 20–
35 mg/day) and the absorption amount (ca. 0.1–0.3 μM) 
of flavonoids in general, it can be suggested that flavonoids 
might act as antagonists of AhR at usual intake levels.

Fig. 8 summarizes the estimated levels and inhibitory 
effects of AhR activation by TCDD of some low-molecular-
weight polyphenolics. Thus, some vegetable polyphenolics 
with low molecular weights and planar structures 
exhibited the properties of agonistic and/or antagonistic 
effects of AhR in the in vitro bioassays, and it can be 
inferrered that they may have an antagonistic function in 
our usual dietary intake.

4. Interactions between some plant food extracts and AhR 
determined by in vitro bioassay

The influences of aqueous alcohol extracts of some plant 
foods on the AhR-signaling pathway were also investigated 
(Amakura et al., 2002, 2004, 2005). The in vitro AhR-
inducing potencies of 39 plant food extracts including 
vegetables, fruits, herbs, and teas were determined by the 
CALUX assay. The induction of luciferase by each extract 
is shown in Fig. 9. Among the vegetables, shiitake (Gie-
bonis coronarius) and spinach extracts induced production 
of luciferase activity of about 50% relative to TCDD maxi-
mum induction at concentrations of 1 mg/ml (plant extract 
dissolved in DMSO), while the other extracts did not
induce luciferase activity (below 10%). Of the fruits, citrus fruits such as grapefruit and lime induced luciferase activity. Among the dried herbs and teas, sage and rosemary induced luciferase activity about 80% and 70%, respectively, at 0.1 mg/ml. On the other hand, in the assay using Ah-I to measure the inhibitory effects of these extracts on AhR activation by TCDD, green leafy vegetables such as spinach and komatsuna, citrus such as orange and grapefruit, and herbs such as sage and peppermint showed marked inhibitory effects (Fig. 10).

The agonistic and antagonistic effects of green leafy vegetables, citrus, and sage, as indicated by the induction of luciferase activity and the inhibitory effect on AhR-induced activation by TCDD, respectively, could be due to the flavonoids and/or related ingredients in these extracts.
5. Concluding remarks

As part of a study on food function and safety, various food polyphenols were assessed for their effects on AhR in relation to toxic dioxins, using in vitro assays of AhR antagonistic and agonistic effects. In the CALUX assay, isoflavones, resveratrol (15), and some flavonoids activated AhR (agonistic effect). Then, a screening of the inhibitory effects of food polyphenols on TCDD-induced AhR activation was conducted using Ah-I, demonstrating that flavones, flavonoids, anthraquinones, coumestrol (36), brevifolin carboxylic acid (34), and resveratrol (15) remarkably inhibited activation (agonistic effect). In addition, aqueous alcohol extracts of consumables such as some green leafy vegetables, citrus fruits, and herbs that are rich in flavonoids were shown to interact with the AhR-signaling pathway in in vitro bioassays (CALUX assay and Ah-I) (agonistic effect at high concentrations and antagonistic effect at low concentrations).

To discuss the utility of food polyphenols for humans, it is necessary to understand not only their functional effects but also the influences of polyphenols on health and safety. Some vegetable polyphenolics with low molecular weights and planar structures exhibited properties of agonistic and/or antagonistic effects of AhR in the in vitro bioassays. However, in light of the bioavailability of such polyphenols, it can be inferred that they may have an antagonistic function in our usual dietary intake. The
AhR for polyphenols in usual intake might function biodefensively to protect the incorporation of foreign chemical compounds such as dioxin. On the other hand, the large excessive intake of foods that contain AhR-activators may be conducive to dioxin-like toxicity, therefore it may be necessary to pay attention to how much of these foods people eat. The results suggest that a well-balanced meal is also important in preventing dioxin-like toxicity.

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References


robenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. Genes Cells 2, 645–654.
Mukai, R., Fukuda, I., Hosokawa, K., Nishimi, S., Kaneko, A., Ahida, H., 2005. Anthocyanins fail to suppress transformation of aryl hydro-
Nishimura, S., Yabushita, Y., Fukuda, I., Mukai, R., Yoshioka, K., Ahida, H., 2006. Molokhia (Cerato1mus litoris L.) extract suppresses trans-
scription of the aryl hydrocarbon receptor induced by dioxin. Food Chem. Toxicol. 44, 259–269.
Pandini, A., Denison, M.S., Song, Y., Soshilov, A.A., Bonati, L., 2007. Structural and functional characterization of the aryl hydrocarbon receptor ligand binding domain by homology modeling and muta-
Safe, S.H., 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environ-
mental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21, 51–88.
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Takashi Yoshida, Ph. D. Professor, Department of Pharmacognosy, College of Pharmaceutical Sciences, Matsuyama University. M.S. in Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kyoto University in 1964; Ph.D. in Pharmaceutical Sciences, Kyoto University in 1969; 1965–1970, Research Associate, Faculty of Pharmaceutical Sciences, Kyoto University; 1970–1993, Associate Professor, Faculty of Pharmaceutical Sciences, Okayama University; 1993–2005, Professor, Faculty of Pharmaceutical Sciences, Okayama University; 2015–Present, Professor, College of Pharmaceutical Sciences, Matsuyama University. He is now also an Emeritus Professor of Okayama University.

Tamio Maltani, Ph.D. Director, Division of Foods, National Institute of Health Sciences (NIHS), Japan. Ph.D. in Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kyoto University in 1979; 1976–1984, Research Scientist and Senior Research Scientist, Division of Basic Medical Sciences, National Institute for Environmental Studies; 1982–1983, Research Fellow, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center (Professor C.D. Klaassen); 1984–1989, Senior Researcher, Division of Foods, NIHS; 1989–2000, Section Chief, Division of Food Additives, NIHS; 2000–2002, Director, Division of Foods, NIHS.