Preliminary Screening of the Inhibitory Effect of Food Extracts on Activation of the Aryl Hydrocarbon Receptor Induced by 2,3,7,8-Tetrachlorodibenzo-p-dioxin

Yoshiaki AMAKURA,*a Tomoaki TSUTSUMI,a Masafumi NAKAMURA,a Hiroko KITAGAWAB,b Junko FUJINOB, Kumiko SASAIB, Takashi YOSHIDAb and Masatake TOYODAb

Division of Foods, National Institute of Health Sciences,a 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan, and Faculty of Pharmaceutical Sciences, Okayama University,b 1–1–1 Tsushima, Okayama 700–8530, Japan.

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A preliminary screening for the inhibitory effects on the activation of the aryl hydrocarbon receptor (AhR) by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by applying AhR-based bioassays for dioxins, the Ah-Immunoassay and CALUX assay, was attempted. Thirty-nine food extracts including vegetables, fruits, herbs, and teas were initially screened in a monitoring method. We first examined the application of both bioassay methods using green tea extracts and (−)-epigallocatechin gallate, reported antagonists of the AhR, since the results could reveal an inhibitory effect versus the control in both assays. Food extracts were then tested. Among the herbs, extracts of sage, among the vegetables, green leafy ones such as spinach, and among the fruit, citrus showed inhibitory effects on AhR activation by TCDD, although some tested samples did not show parallel behavior in both assays. Sage had a remarkable inhibitory effect (79% in the CALUX assay and 83% in the Ah-Immunoassay compared with control) and its effects were dose dependent. The results suggest that these assays might be applicable to the preliminary screening of antagonist activity against the AhR. Moreover, based on these results, the potential benefit of factors that function as dietary ligands of the AhR and are present in several foodstuffs is indicated.

Key words food; aryl hydrocarbon receptor; dioxin; CALUX assay; Ah-Immunoassay

The aryl hydrocarbon receptor (AhR), also called the dioxin receptor, is a ligand-activated transcription factor that exists in most cell and tissue types of the body.1) Potent xenobiotic ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which are archetypal dioxins known as the most potent congener of a family of dioxin-type chemicals, enter cells and bind to a protein in the AhR. After further exchange of some smaller proteins (AhR translocator, ARNT), this ligand-AhR complex becomes transformed, enters the nucleus, and binds to a specific dioxin receptor element (DRE) of DNA. This DNA interaction occurs upstream from genes that are then activated to produce certain enzymes, some of which are highly correlated with subsequent toxicity events including carcinogenicity, developmental and reproductive toxicity, and immunological impairment.2–7) Accordingly, AhR activation by the binding of dioxins is assumed to be one of the first and key steps in the development of dioxin toxicity. The inhibition of AhR activation could be expected to provide protection against such toxicity. Human exposure to dioxin has been proposed to occur predominantly through foods such as fish, meat, dairy products, and some vegetables.8) Therefore if factors that inhibit AhR activation are present in our daily diet, they might play a role in protection against dioxin toxicity, consequently leading to a lower risk to human health.

Recently, several studies have examined the effects of dietary components such as flavonoids, etc., on the binding of TCDD to AhR, and it was found that these components can exert an antagonistic activity.9–11) The effects of green tea and its catechins, have also been examined, and the results indicated that they can compete with TCDD for binding to the AhR.12) However, studies on foodstuffs themselves are limited.

Based on this background information, we attempted to apply two AhR-based bioassays for dioxins, the Ah-Immunoassay (Ah-I) and the CALUX assay, in this preliminary screening of foods to determine their inhibitory effect on AhR activation caused by TCDD. The results indicated that certain foods might be potential inhibitors of AhR activation.

We postulated that the inhibition of AhR activation by TCDD could be assessed by the Ah-I (Kubota, Osaka, Japan) and CALUX bioassay methods using green tea extracts and (−)-epigallocatechin gallate, which have been identified as antagonists of the AhR. The Ah-I method is a receptor-binding assay using cytosol containing AhR extracted from mammalian liver cells, which immunologically measures the dioxin level utilizing the antigen-antibody reaction. On the other hand, the CALUX assay uses a patented recombinant murine cell line that contains the luciferase reporter gene under the control of dioxin-responsive elements.13) When these cells are exposed to environmental ligands such as dioxins, the luciferase protein is synthesized. The amount of light emitted by the luciferase protein is directly correlated with the dioxin level, and this is used as a simple dioxin monitoring method.

In the present study, the Ah-I was employed as follows. The cytosol (200 μl) was added to the sample at final concentrations of 50 μg/ml or 50 μM, or dimethyl sulfoxide (DMSO) alone was added as the control, the mixture was preincubated for 10 min, and then incubated with 5 nM TCDD for 2 h (the final DMSO concentration was 1% in cell culture medium). After incubation, the formation of the TCDD-AhR complex was determined with an ELISA kit and the absorbance was determined at 405 nm using a microplate reader.

In the CALUX assay, mouse hepatoma H1L1 cells (ca. 1.5×10³ cells/well) were cultured in 96-well culture plates and samples at final concentrations of 25 μg/ml or 25 μM dissolved in DMSO were added to the medium 10 min prior to incubation with 1 nM TCDD using DMSO as the vehicle (the final DMSO concentration was 1% in cell culture medium). The plates were incubated at 37 °C in 5% CO₂ for 20 h to produce the optimal expression of luciferase activity. After incubation, cell viability was confirmed under a microscope. Subsequently, the medium was removed and the cells were lysed. After the addition of luciferin as the substrate, the light emitted by the luciferase protein is directly correlated with the dioxin level, and this is used as a simple dioxin monitoring method.

* To whom correspondence should be addressed. e-mail: amakura@nihs.go.jp © 2002 Pharmaceutical Society of Japan
control with TCDD added, $B$ is the absorbance (or RLU) of the control with DMSO added, $C$ is the absorbance (or RLU) of the sample solution with TCDD added, and $D$ is the absorbance (or RLU) of the sample solution with DMSO added. All experiments were carried out in triplicate. The values obtained with DMSO alone were considered as 100% of the control value.

Figure 1a shows the results for green tea extracts and (−)-epigallocatechin gallate. Both decreased the AhR binding activity or luciferase activity compared to the control. These results suggest that these bioassays might be applicable to the preliminary screening for inhibition of AhR activation. They could also be employed for water-soluble samples, because the two tested samples are soluble in water.

Thirty-nine dietary vegetables, fruits, herbs, and teas were arbitrarily selected, and their extracts were evaluated for their inhibitory effects on AhR activation using the Ah-I and CALUX assay. The tested fresh vegetables and fruits were purchased from local grocery stores. The dried herbs and teas were donated by Nagaoka Perfumery (Osaka, Japan). The samples were prepared using an approved method as follows: the materials (50 g of fresh and 10 g of dried materials) were homogenized in 50% aqueous ethanol (100 ml), and then the homogenates were filtered. The filtrates were
concentrated under reduced pressure and freeze-dried. The extracts were dissolved in DMSO and then evaluated for their inhibitory effects on AhR activation. Figures 1b and 2 show the effects of the tested samples on AhR activation by TCDD using both assays. Of the tested herbs and teas, the sage extract had a marked inhibitory effect in both assays (79% in the CALUX assay and 83% in the Ah-I compared with control). Among the tested vegetables and fruits, the green leafy vegetable extracts such as spinach and citrus extracts such as lime had greater inhibitory activity than the control (Fig. 2). Figure 3 depicts the inhibitory effects of several concentrations of sage, lime, and spinach extracts evaluated by the CALUX assay. These sample extracts dose-dependently suppressed TCDD-induced AhR activation. This antagonistic effect might be due to the flavonoids and/or related ingredients in these extracts.

When the results of the two assays were compared, some tested samples did not show similar activity in both. For example, while lime extract had similar inhibitory effects in both assays (49% in the CALUX assay and 50% in the Ah-I compared with control), whereas the extracts of lemon, komatsuna, etc., were not equivalent (lemon, 25% in the CALUX assay and 61% in the Ah-I; komatsuna, 28% in the CALUX assay and 87% in the Ah-I). Although this discrepancy cannot be fully explained at this stage, the Ah-I is a receptor-binding assay that detects the reactivity of AhR combined with dioxins on an ELISA plate without using living cells and the CALUX is a bioassay that assesses dioxins via in vitro activation of the AhR of cultured mouse hepatoma H1L1 cells. This difference in the detection method might have influenced the present results. In addition, the dioxin level in the body is usually much lower than the tested concentrations of 1 or 5 nM, which were appropriate concentrations for detection by each assay. Therefore the results of this study should be considered to reflect general trends. Further studies to investigate these effects in more detail are planned.

The present results show that several food extracts may prevent dioxin toxicity associated with the AhR using our preliminary methods. These results with foodstuffs open a new frontier in the search for agents that can protect against dioxin toxicity.

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REFERENCES


Fig. 3. Dose-Dependent Inhibitory Effect of Sage, Lime, and Spinach Extracts on AhR Activation by TCDD 1 nM in the CALUX Assay
The data points represent the mean±S.D.