DETERMINATION OF DIOXINS IN EMISSION GAS, ASH AND ATMOSPHERIC AIR SAMPLES USING A HIGHLY SENSITIVE AHR-MEDIATED REPORTER CELL LINE, DR-ECOSCREEN CELLS

Kojima H[1], Takeuchi S[1], Anezaki K[2], Yamaguchi K[2], Otsuka H[2],
Sawadaishi K[3], Honda K[3], Yabushita H[4], Iida M[4]

[1]Hokkaido Institute of Public Health, Sapporo, 060-0819 Japan; [2]Hokkaido Institute of Environmental Sciences, Sapporo, 060-0819 Japan; [3]Ehime University, Matsuyama, 790-8566 Japan; [4]Otsuka Pharmaceutical Co., Ltd., Tokushima 771-0195, Japan

Abstract

There is a strong need for the development of relatively low-cost and rapid bioassays for the determination of dioxins and related compounds in environmental and food samples. We have established the aryl hydrocarbon receptor (AhR)-mediated reporter cell line (DR-EcoScreen cells), which highly expresses the luciferase gene and can be adapted for high-throughput machinery. The bioassay using DR-EcoScreen cells showed a high sensitivity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and the relative effect potencies of 29 dioxins (PCDD/Fs and dioxin-like PCBs) obtained using this assay showed close correlation to the toxic equivalency factors (TEFs) proposed by WHO. In order to test if the DR-EcoScreen bioassay is suitable for the screening of dioxins, we compared the DR-EcoScreen bioassay with the high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) method for emission gas, ash (fly and bottom) and atmospheric air samples. As a result, the toxic equivalency (TEQ) values obtained by DR-EcoScreen bioassay correlated well with the TEQ values obtained by HRGC/HRMS analysis (r = 0.950 for emission gas, r = 0.940 for ash, and r = 0.801 for atmospheric air). These data clearly indicate that the DR-EcoScreen bioassay might be a promising method for the rapid and low-cost screening of dioxins in various field samples.

Introduction

High-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is currently the most widely used method for determining dioxin concentrations. However, this method requires expensive equipment and highly trained analysts, whilst the sample preparation procedures are often time consuming and costly. Therefore, the development of a rapid and inexpensive screening method for dioxins is of high priority. A number of studies have reported bioassay systems, such as the chemical-activated luciferase gene expression (CALUX) assay, as possible alternatives for screening dioxins[1-4]. CALUX assay can detect dioxin-like compounds based on their activation of the aryl hydrocarbon receptor (AhR), which increases expression of the luciferase reporter gene. Consequently, biological approaches offer a number of advantages such as low costs, rapidity, small sampling and so on.

Recently in our laboratory, a genetically engineered stable cell line, designated DR-EcoScreen, has been

developed as a sensitive and rapid method for screening AhR agonists among a number of environmental chemicals[5]. The bioassay using DR-EcoScreen cells has greater sensitivity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and more simple procedure as compared to other bioassays. In this study, we applied the DR-EcoScreen bioassay to the determination of dioxins in environmental samples, such as emission gas, ash and atmospheric air, and confirmed the DR-EcoScreen bioassay as a rapid and inexpensive screening method for dioxins through comparing with the HRGC/HRMS method.

Material and Methods

Chemicals and samples. Standard samples of 29 dioxins were used for comparison with WHO-TEFs. The extracts of emission gas (n = 31), fly and bottom ash (n = 44), and atmospheric air (n = 32) samples were used as environmental matrices for comparison between the DR-EcoScreen bioassay and HRGC/HRMS analysis.

Clean-up of sample extracts. For DR-EcoScreen bioassay, the extracts of emission gas and ash were cleaned up using an automated-sample preparation device, SPD-600[6] (Kyoto Electronics Manufacturing Co., Ltd.). Atmospheric air samples were passed through a multilayer silica gel column, and an activated carbon column connected to a high performance liquid chromatograph. These samples were finally dissolved in DMSO for measurement. For HRGC/HRMS analysis, three kinds of sample extracts were subjected to clean up procedure with multilayer silica gel and activated carbon column chromatography.

HRGC/HRMS chemical analysis. A high-resolution gas chromatograph (HRGC) coupled to a high-resolution mass spectrometer (HRMS) was used for analysis and detection of 17 PCDD/Fs and 12 dioxin-like PCBs.

DR-EcoScreen bioassay. A mouse hepatoma Hepa1c1c7 cell line was stably transformed with a plasmid containing dioxin-responsive elements (DRE) fused to a luciferase gene, thus establishing DR-EcoScreen cells[5]. The DR-EcoScreen cells were suspended with alpha-MEM medium supplemented with 5 % fetal bovine serum and antibiotics, and plated in 96-well microtiter plates at a density of 9,000 cells per well. After 24 h, various concentrations of compounds, cleaned up samples, 2,3,7,8-TCDD, or 1% DMSO (vehicle control) in complete medium were administered to the cells. After an incubation period of 24 h, the luciferase substrate Steady-Glo™ reagent (Promega) was added to the cells, and the luciferase activity was measured using a Wallac 1420 ARVO™ SX multi-label counter (Perkin-Elmer). The responses for samples containing dioxin-like compounds were converted into 2,3,7,8-TCDD toxic equivalency (TEQ) using a 2,3,7,8-TCDD standard curve.

Results and Discussion

The DR-EcoScreen cells were treated with various concentrations of 2,3,7,8-TCDD for 24 h. As shown in Fig.1, this bioassay was 2,3,7,8-TCDD dose dependent, and could detect the AhR agonistic activity of 2,3,7,8-TCDD from a very low concentration (0.2 pg/ml) with small variance of data (within CV 10%). This indicates that the DR-EcoScreen bioassay is highly sensitive and responsive to 2,3,7,8-TCDD, and is able to detect a wide range of concentrations from 0.2 pg/ml to 10 pg/ml 2,3,7,8-TCDD.

Based on the chemical analysis of each congener of the selected 29 dioxin-like compounds, the TEQ values

can be calculated as the sum of the multiples of concentration of each congener with WHO-TEF values. Therefore, we conducted dose-response studies of the 29 dioxins by DR-EcoScreen bioassay, calculated the relative effect potency (REP), and compared those with the WHO-TEF values. As a result, the REP values obtained from the DR-EcoScreen bioassay showed close correlation to the WHO-TEF values (data not shown).

In order to confirm whether the DR-EcoScreen bioassay can precisely predict the amount of dioxin-like compounds, we measured TEQ values in environmental samples such as emission gas, ash and atmospheric air using the DR-EcoScreen bioassay as well as HRGC/HRMS analysis. As shown in Fig.2 A and B, there were close correlations between the TEQ values obtained by the two methods on emission gas and ash samples (r = 0.950 and r = 0.940, respectively). In addition, we also confirmed that the TEQ values obtained by the two methods were well correlated on very low concentration samples such as atmospheric air (r = 0.801), thereby demonstrating that our DR-EcoScreen bioassay is highly sensitive (Fig.2 C). On the other hand, TEQ values from the bioassay were more fivefold than those from HRGC/HRMS. The reason for this discrepancy may reflect the differences between the DR-EcoScreen-REP and WHO-TEF values for the 29 dioxins and, moreover, seems to be associated with the ability of the DR-EcoScreen bioassay to recognize all dioxin-like compounds with or without assigned WHO-TEF values. For example, some of the PCDD/Fs, polybrominated dibenzo-*p*-dioxins/furans (PBDD/Fs) and polychlorinated PAHs without assigned WHO-TEF values have been reported to possess AhR agonistic activity. As such compounds are present even in the cleaned-up samples, it is speculated that they contribute to TEQ values obtained by the DR-EcoScreen bioassay.

Besides high sensitivity, the DR-EcoScreen bioassay has unique advantages as compared to other bioassays. As the DR-EcoScreen cells have very strong luminescence intensity and can be measured using a long lived luciferase substrate, the bioassay using this cells dose not need the process of well-washing and medium changes in all procedure. Therefore, the DR-EcoScreen bioassay is compatible to the high-throughput automation and can reduce the overall workload in laboratory. Taken together, these results indicate that the bioassay using DR-EcoScreen cells is a promising method for the sensitive, rapid and low-cost screening of dioxins in various field samples including foods.

References

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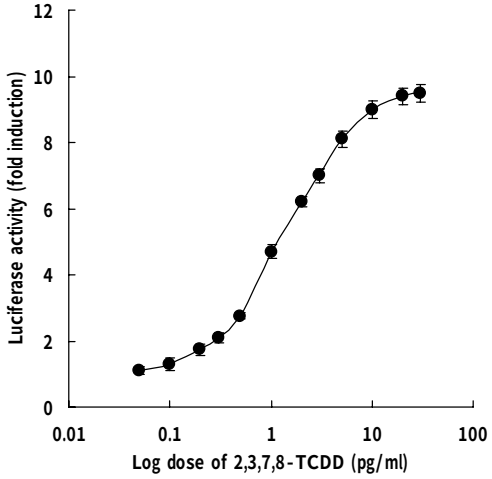

Fig. 1 Dose-response curve for induction of luciferase activity by 2,3,7,8-TCDD in the DR-EcoScreen bioassay. DR-EcoScreen cells were treated with 1% DMSO (vehicle control) or various concentrations of 2,3,7,8-TCDD for 24 h. The luciferase activity was normalized to cell viability and expressed as a fold induction over that of control. The EC50 of 2,3,7,8-TCDD was calculated to be 1.2 pg/ml.

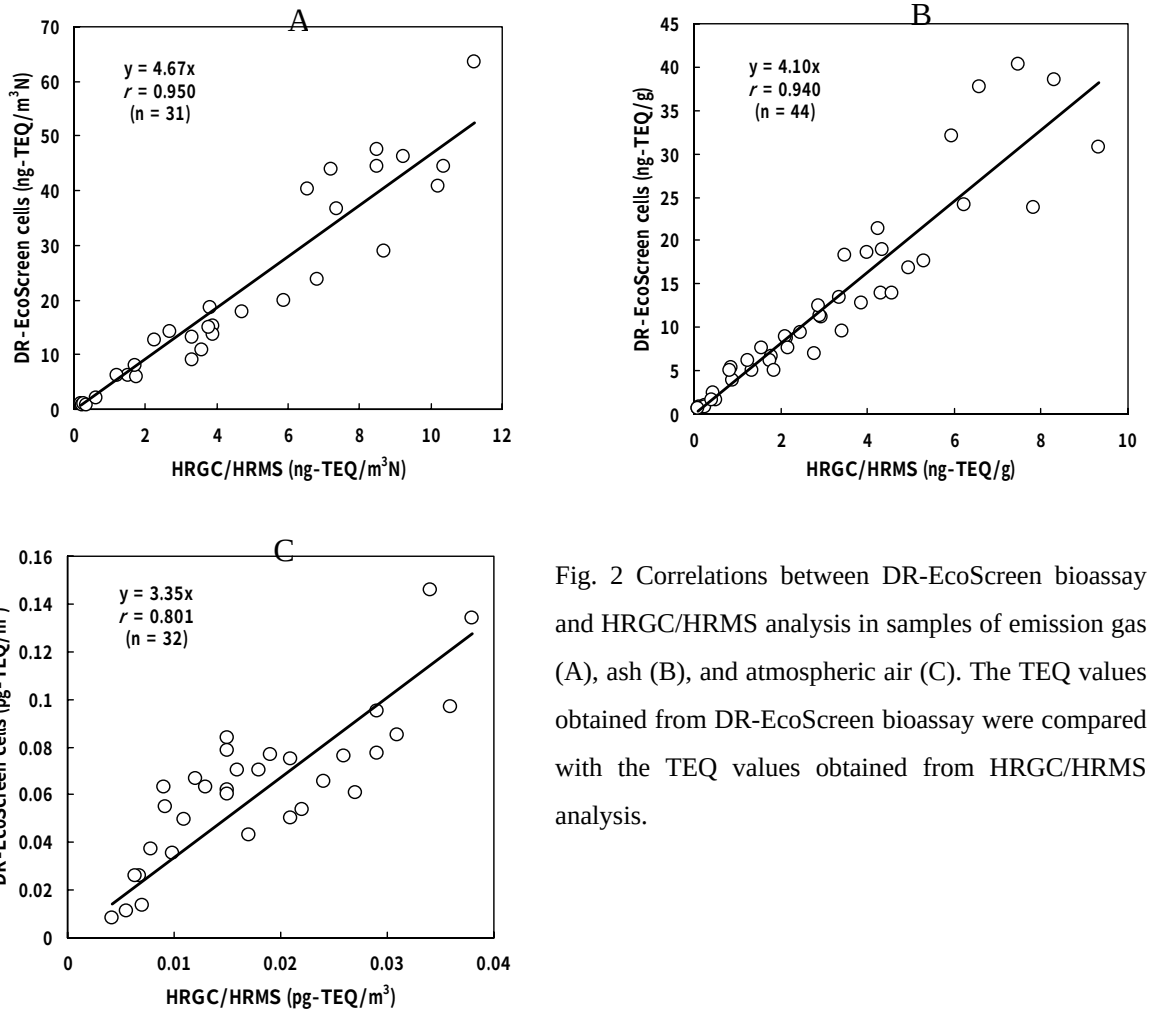

Fig. 2 Correlations between DR-EcoScreen bioassay and HRGC/HRMS analysis in samples of emission gas (A), ash (B), and atmospheric air (C). The TEQ values obtained from DR-EcoScreen bioassay were compared with the TEQ values obtained from HRGC/HRMS analysis.