DEVELOPMENT OF DIOXIN TOXICITY EVALUATION METHOD IN HUMAN MILK BY ENZYME-LINKED IMMUNOSORBENT ASSAY (PART V: A STUDY ON IMPROVEMENT OF STABILITY)

Masahiro Ishizuka[1], Yukio Sugawara[1], Koichi Saito[2], Mikiko Takekuma[2], Masahiko Ogawa[2], Susumu Kobayashi[2], Guomin Shan[3], Bruce D. Hammock[3], Hiroyuki Nakazawa[4], and Yasuhiko Matsuki[5]

[1] Cosmo Research Institute, 4-9-25, Shibaura, Minato-ku, Tokyo 108-8564, Japan
[2] Dioxin Research Group, Saitama Institute of Public Health, 639-1, Kamiokubo, Urawa, Saitama 338-0824, Japan
[3] Department of Entomology, University of California, Davis, CA 95616, U.S.A.
[4] Department of Analytical Chemistry, Hoshi University, 2-4-41, Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[5] Food and Drug Safety Center, Hatano Research Institute, 729-5, Ochiai, Hadano, Kanagawa 257-8523, Japan

Introduction

The social concern to the contamination of the ecosystem and food by dioxins and to the risk of human health is very high. It is an urgent and an important task for the government to grasp exposure to human in actual contamination of dioxins. In dioxin analysis, the establishment of an appropriate and an accuracy method for the measurement is indispensable for reliability. In addition, we need simple system, which is able to analyze rapid and mass screening. The purpose of this research is establishing ELISA for measuring dioxins in human samples, which is easy, rapid, and highly sensitive to perform, and contributing to monitor of investigations for pollution and actual conditions by dioxins. We reported the development of dioxin toxicity evaluation method in human milk by ELISA at this symposium of last year [1-3]. Since we have developed the dry type plates in order to put in practical use our ELISA system that we constructed last year, we reported its basic performance such as cross-reactivity and the correlation with the GC/MS values by new our ELISA system using the dry plates.

Experimental Methods

1. Development of the Dry Plates

Microtiter plates were coated with the optimized concentration (0.5μg/mL, 100 μL/well) of coating antigens (Hapten Ⅲ)[4] in carbonate-bicarbonate coating buffer (pH 9.6). They were incubated overnight at 4 °C. The following day, the coated plates were washed 5 times with 0.05% (v/v) Tween 20 in PBS and were incubated for 30 min at room temperature with 300 μL of a 0.5% (w/v) BSA with sucrose in PBS (blocking solution) per well. After the removal of the blocking solution, the plates were dried *in vacuo* for 4 hours at 25 °C. And they were put into the aluminum bags respectively, and were packed *in vacuo*. They were checked the stability test periodically at 2-11°C (regular stability test), and at 37 °C (accelerated stability test).

2. Determination of Cross-reactivities

We evaluated the cross-reactivity of the ELISA about 27 kinds of dioxin congeners, which contribute 17 PCDD/F congeners and 3 Co-PCB congeners to the toxicity equivalents (TEQ), and 7 congeners with less than four chlorine atoms, using the dry plates. The 27 kinds of dioxin congeners (*50 μg/mL in nonane*) were diluted 10-fold with decane respectively. 0.25mL of each diluted solution was evaporated to dryness under nitrogen and then redissolved into 250 μL of DMSO with 100 ppm Triton X-100 for the ELISA under 5-min sonication. The solutions were serially diluted to 0.0128 pg/well with DMSO with 100 ppm Triton X-100 and applied for the ELISA which method has reported previously[3]. The cross-reactivities (CR) were calculated relative to the concentration producing 50% inhibition (IC_{50}) by 2,3,7-trichloro-8-methyldibenzo-*p*-dioxin (TMDD). The data were obtained from standard curves of the related compounds and calculated according to the following formula: %CR =(IC_{50} of TMDD/IC_{50} of the cross-reacting compound) x100

3. Comparison of a Commercial Kit and our ELISA System

We measured the dioxin in human milk using the commercial kit and our ELISA system. The sample was prepared according to the method that we reported previously[1,2]. The alkali decomposition was performed after fat extraction from human milk, and then the sample was cleaned up by the three-layer sulfuric acid silica gel column.

Results and Discussion

1. Performance of the Dry Plate

Figure 1 shows the representative standard curves to TMDD using the dry plates and the conventional wet plate. The standard curve of the dry plates was showed almost the same as that of the wet plates. The IC_{50} in the standard curve of the wet plates was 27.1 pg/well, whereas the IC_{50} in that of the dry plates was 19.8 pg/well, and the sensitivity of the dry plates was slightly higher than that of the wet plates. Regarding stability, degradation of the dry plates was not observed for one month by accelerated stability test at 37 °C. It is equivalent to the ability to keep this for one year at 4 °C. It turns out that the dry plates can be used in practical.

2. Cross-reactivity

For determination of cross-reactivity, the IC_{50} of TMDD was assigned a value of 100%, and the cross-reactivities for other compounds were reported according to their IC_{50}'s relative to this value. 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD have higher cross reactivities than other compounds with both plates (not shown).

Moreover, good correlation was acquired by plotting cross-reactivity obtained for each compound on the vertical (Y) axis versus the corresponding TEF on the horizontal (X) axis (Figure 2). As this result, it has shown the antibody used by this study had not only the excellent specificity against 2,3,7,8-TCDD, but the practicability as a method of screening for evaluating the toxicity based on TEQ.

3. Comparison of a Commercial Kit and our ELISA System

In order to examine the practicability, we measured the dioxin in human milk using the commercial kit and our ELISA system. Consequently, the commercial kit did not display significant correlation to GC/MS. Whereas in our ELISA system, a good correlation between ELISA values and TEQ

(total-TEQ) values was observed with human milk (y=0.422x-1.353, r=0.902, n=17). Originally, the commercial kits were developed for measurement of dioxins in environmental samples such as fly ash, soil, and chimney soot, which contained dioxins in high concentration. Therefore, there has been no report on a practical assay that can deal with biological samples containing dioxins in extremely low concentration such as human milk and blood. Whereas as we reported previously, our ELISA system has been developed to be able to measure a low concentration of dioxins like those in human milk, and we showed it. At this time, although we were changed our plate system from the wet plates to the dry plates to seek the more practicability, we have not only obtained almost the same performance as the results using the dry plates but also have improved the stability. Thus this ELISA system with the dry plates indicated usefulness as a toxicity evaluation method for dioxins in human milk.

Acknowledgement

This project was supported in part by Health Sciences Research Grants from the Ministry of Health and Welfare of Japan and NIEHS Superfund Research Program, 5 P42 ES04699-08, the NIEHS Center for Environmental Health Sciences and USEPA Center for Ecological Health Research CR819658(U.S.A.).

Reference

1. Nakazawa, H., Sugawara, Y., Saito, K., Ogawa, M., Kobayashi, S. and Matsuki, Y. (2000), Organohalogen Compounds, 45, 176-179.

2. Saito, K., Ogawa, M., Takekuma, M., Kobayashi, S., Sugawara, Y., Nakazawa, H. and Matsuki, Y. (2000), Organohalogen Compounds, 45, 168-171.

3. Sugawara, Y., Saito, K., Ogawa, M., Kobayashi, S., Shan, G., Hammock, B.D., Nakazawa, H. and Matsuki, Y.(2000), Organohalogen Compounds, 45, 172-175.

4. Sugawara, Y., Gee, S.J., Sanborn, J.R., Gilman, S.D. and Hammock, B.D. (1998) Anal. Chem. 70, 1092-1099.

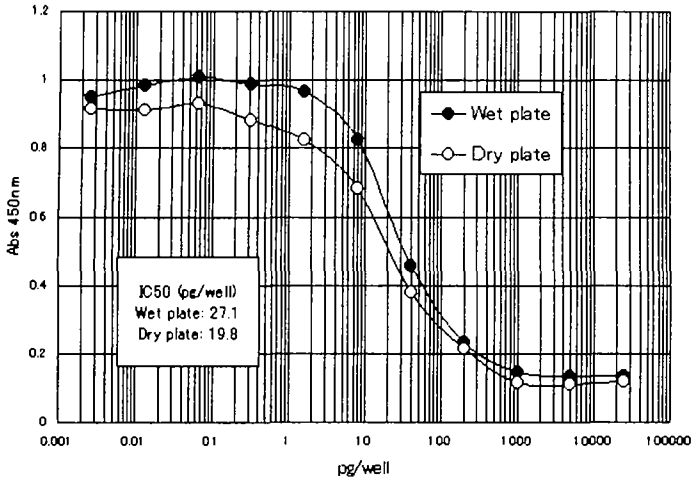

Figure 1. Standard curves to TMDD using the dry plates and the wet plates.

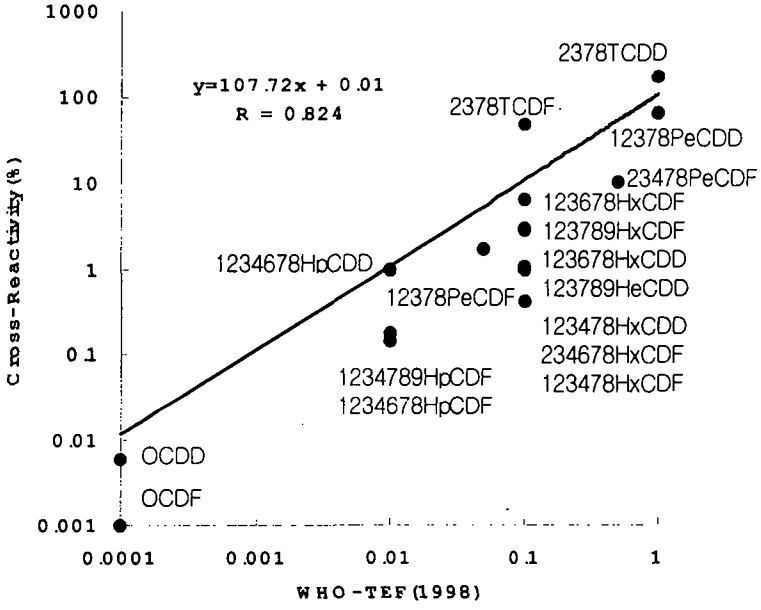

Figure 2. Relationship between Cross-reactivity of our ELISA system and WHO-TEF