Development of Dioxin Toxicity Evaluation Method in Human milk by Enzyme-Linked Immunosorbent Assay (part I: Basic Strategy for Methodology Construction)

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Introduction

Contamination of food and the ecosystem by dioxins and its resultant effects on our health have been drawing much attention from the public. Thus, to investigate the exposure of humans to dioxins is an urgent and important task for the government. Since Law Concerning Special Measures against Dioxins became effective in Japan in 1999, the numbers of substances to be measured and samples are expected to increase.

Conventionally, high resolution gas chromatography/mass spectrometry (GC/MS) has been used for measurement of dioxins. However, GC/MS method has a drawback in that it requires a complicated clean-up procedure for measurement of all types of samples as well as it is very time-consuming and extremely expensive to perform. Thus, the development of a method for measuring dioxins, which is inexpensive, easy to perform, and highly sensitive, has been highly demanded by the public and the government. One of the methods that may satisfy the requirements is an enzyme-linked immunosorbent assay (ELISA), and there have been some reports on measurement of dioxins by use of ELISA. However, most of the reports dealt with standard substances[1,2], fly ash[3], soil[4,5], and chimney soot[6], which contained dioxins in high concentration. 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. The conventional ELISA has been considered as a simple screening method, and to be less reliable compared with GC/MS method. In this report, we constructed a basic strategy for the development of a toxicity evaluation method for dioxins in human milk by ELISA. Also, we selected an optimal isomer to be detected, which is the key factor to the construction of ELISA.

Experimental Methods

1. Overall approach

We consider that an ELISA suitable for measurement of dioxins in biological samples should satisfy the following requirements:

1) The ELISA should be able to evaluate toxicity directly instead of providing only a simple screening method,

2) The ELISA should share a common pre-treatment procedure with the conventional GC/MS method so that valuable biological samples are effectively used, and also be compatible with the GC/MS. Moreover, the ELISA should not require a complicated pre-treatment procedure.

3) The data obtained from actual samples (human milk) by the ELISA should be highly correlated with those obtained by GC/MS method.

In this report, we will focus on 1), and reports on 2) and 3) (part II and part III) will be given in other reports in this symposium.

2. Study of an isomer to be targeted for the construction of a toxicity evaluation method

Since ELISA has a property that it can perform a specific measurement only on a specific chemical substance, it cannot separate or determine different isomers unlike GC/MS method. However, by use of an antibody that is highly responsive to an isomer having a high toxicity and poorly responsive to an isomer having a low toxicity, the results by ELISA provide not only detected values but also degrees of toxicity. In order to develop a toxicity evaluation method by ELISA based on this concept, it was necessary to examine the relationship between various isomers detected from biological samples and their toxicity equivalents (TEQ). Accordingly, 100 samples of human milk were analyzed by GC/MS method to find isomers that are highly correlated with TEQ and detected in large amounts.

Dioxins in human milk were measured in the following manner: According to a conventional method, fat was extracted from human milk, and the fat was subjected to sulfate treatment, and then to various chromatographies (silica gel, alumina, and activated charcoal silica gel), followed by measurement by GC/MS. DB-17HT was used as a separation capillary column in GC, and JEOL JMS-700 was used as a high resolution MS. SIM was measured with the resolution being set at 10000. The concentrations of dioxin isomers were calculated on fat basis, and the TEQs were calculated based on WHO-TEF (1998).

Results and Discussion

In this study, we constructed a basic strategy for the development of a highly sensitive and simple method for measurement of dioxins in human milk by ELISA and for the evaluation of the method as a toxicity evaluation method. We presented ELISA as a method for evaluating toxicity directly not as a simple screening method. This is a new approach to ELISA. Fig. 1 shows a flow chart of the concept of an assay aimed in this study. As shown in Fig. 1, after fat is extracted from human milk, a simple pre-treatment (Step I) is performed on the fat, and then part (half) of the pre-treated fat as a testing solution is immediately evaluated for its toxicity by ELISA. If the concentration of each isomer needs to be measured or the TEQ needs to be confirmed, the results obtained by ELISA are feed-backed, and the remaining samples are subjected to clean-up (Step II), followed by measurement by GC/MS method. This method is considered to increase the additional value of the data obtained by ELISA and enable ELISA to be compatible with GC/MS method.

We also examined isomers to be targeted for the construction of a toxicity evaluation method of dioxins in human milk. One hundred samples of human milk were analyzed by GC/MS method and the correlation between the obtained dioxin isomers and the TEQ were examined. As shown in Fig. 2, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF were found to be highly correlated with the TEQ ($r=0.962$, $r=0.941$, respectively). The conceivable reason for this is that both have high toxicity equivalent factors (TEF) and are detected in relatively large amounts. In contrast, a typical dioxin isomer, 2,3,7,8-TCDD was found to be correlated to the TEQ ($r=0.759$), but not as highly as the above two isomers. This is probably due to a smaller amount of detected 2,3,7,8-TCDD. OCDD

had the highest concentration among isomers detected from human milk, but showed a low correlation with the TEQ (r=0.421) because of its extremely low TEF of 0.0001. Based on these results, the optimal isomers to be targeted for measurement by ELISA were found to be 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF.

Acknowledgement
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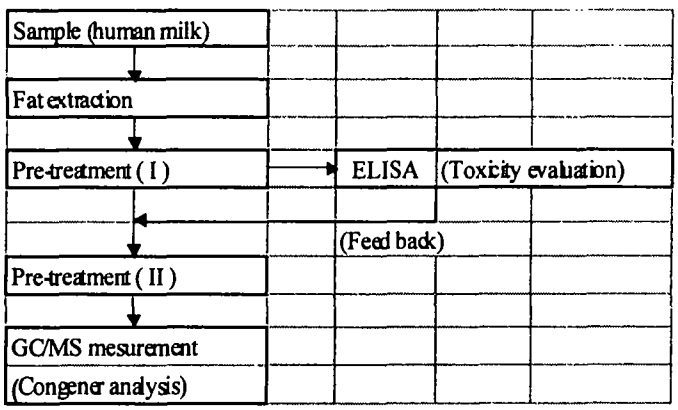

Figure 1. Flow chart of dioxin analysis in human milk by ELISA and GC/MS method

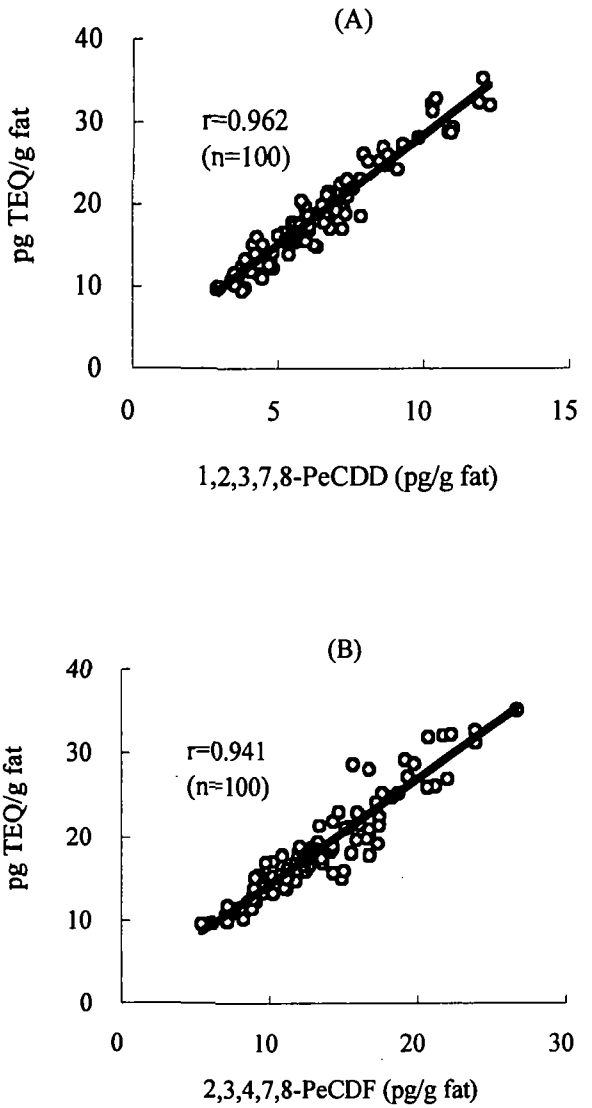

Figure 2. Correlation between a dioxin isomer and TEQ;
(A) 1,2,3,7,8-PeCDD and TEQ, (B) 2,3,4,7,8-PeCDF and TEQ.