Enzyme-Linked Immunosorbent Assay for Dioxins Based on Monoclonal Antibodies

Mitsunobu Okuyama[1], Wakako Endo[1], Takako Anjo[1], Akira Kambegawa[2], Norihiro Kobayashi[3], Junichi Goto[3], Yasuhiko Matsuki[1]

1 Food & Drug Safety Center, Hatano Research Institute, 729-5, Ochiai, Hadano, Kanagawa 257-8523, Japan

2 Kambegawa Research Institute, 3-8-5, Motoizumi, Komae, Tokyo 201-0013, Japan

3 Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai 980-8578, Japan

Introduction

Environmental chemical contaminants have been concerns in the public and the government for many years. Particularly, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are persistent environmental pollutants with potential diverse toxic, teratogenic, reproductive, immunotoxic and carcinogenic effects, because of their high lipophilicity. Therefore, PCDD/Fs are subjects of surveillance by regulatory agencies. Conventionally, high-resolution gas chromatography/mass spectrometry (GC/MS) has been used for reference method equipping enough specificity and sensitivity to assess health risks that may be related to exposure to PCDD/Fs. However, GC/MS method is available in relatively limited research institute, and furthermore it requires time-consuming sample clean-up procedures, use of refined laboratories and costly analytical equipment with skilled operators, because of the minute amount in environmental and biological matrices. Thus, a quick and easier method, which is suitable for routine analysis of PCDD/Fs, is in demand for large-scale epidemiological study or long-term monitoring. Immunoassay could be a desirable method with enough specificity, sensitivity and feasibility. Several enzyme immunoassay procedures for PCDD/Fs have been reported, but most of them required a large amount of standard or environmental samples[1-8]. We previously established an enzyme-linked immunosorbent assay (ELISA) for PCDD/Fs using rabbit

anti-dioxin antisera and horseradish peroxidase (HRP)-labeled haptens, whose assay values were in highly sensitive and good correlation to TEF[9]. In this study, we established mouse monoclonal antibodies against PCDD/Fs and developed an ELISA system using one of the antibodies ,which would be expected method for routine analysis.

Materials and Methods

1. Chemicals and immunoreagents

PCDD/Fs congeners were purchased from Wellington Laboratories. Rabbit anti-mouse IgG + IgM and Triton X-100 were obtained from Jackson ImmunoResearch Laboratories, Inc. and Sigma Chemical Co., respectively. Four kinds of dioxin haptens, corresponding bovine serum albumin (BSA) conjugates and HRP-labeled antigens were synthesized as previously reported[9].

2. Immunization and monoclonal antibody production

Female BALB/c and A/J mice (8 weeks of age) were immunized with the BSA conjugates of haptens shown in figure 1 at approximately 3-week intervals. The immune spleen cells and P3/NS1/1-Ag4-1 myeloma cells were fused as described previously[10].

3. ELISA

A solution of the second antibody (1μg/mL) in phosphate buffered saline（PBS）, pH 7.4, was distributed in each well of 96-well ELISA plates (100μL/well), which were left overnight at 4℃. After washing twice with PBS, the wells were blocked with a 0.5% BSA solution in PBS (200μL) at room temperature for 2 h. The wells were washed twice with PBS, to which HRP-labeled hapten (20ng; 50μL), adequately diluted monoclonal antibody and standard PCDD/Fs or biological sample each diluted with PBS containing 0.1% gelatin and 0.02% Triton X-100 (50μL) were added. After overnight incubation at 4℃, the solution were discarded and the wells were washed 3 times with PBS. Then a substrate solution (100μL; 50 mmol/L citric acid-acetate buffer, pH 5.0, containing 0.01% H_2O_2 and 0.05% *o*-phenylenediamine) was distributed and the plates were incubated at room temperature for 1 h. After addition of 3 mol/L sulfuric acid (50μL) to terminate the emzymic reaction, the absorbance at 490 nm was measured by a microplate reader (BL 312e, Bio-Tek Instrument Inc.).

Results and Discussion

Among 12 kinds of antibody-secreting hybridomas (obtained from 5 fusion experiments), we selected three hybridoma lines (D2-37, D9-36, D35-42), which had been producing characteristic anti-dioxin antibodies. Using the monoclonal antibodies D2-37 and D9-36 in the combination with the labeled antigen, I-5-HRP, the standard curves for 2,3,7,8-TCDD were obtained with satisfactory sensitivity. Furthermore, the cross-reactivity of these two monoclonal antibodies to dioxin congeners was in a good correlation with TEF values. The dose-response curve with D35-42 was less sensitive than that with D2-37 or D9-36, but this antibody equipped a useful feature that it showed high specificity toward the most toxic congeners, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD.

The concentration of Triton X-100 in the assay buffer affected the sensitivity for PCDD/Fs, suggesting that the detergent plays an important role on solubilizing PCDD/Fs in aqueous medium.

We have established an ELISA for PCDD/Fs using the newly generated monoclonal antibodies, which could be a standard assay method. By combination with a rapid, simple and reproducible clean-up procedure, this ELISA method could be widely applicable to various environmental and biological matrices.

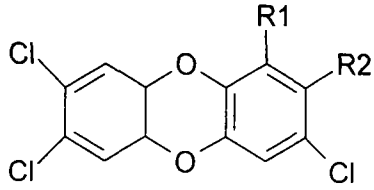
Acknowledgments

This work was supported in part by the Health Science Research Grants from the Ministry of Health and Welfare of Japan.

References

1. Stanker,L.H., Watkins,B., Rogers,N., Vanderlaan,M. (1987) Toxicology **45**, 229-243
2. Vanderlaan,M., Stanker,L.H., Watkins,B.E., Petrovic,P., Gorbach,S. (1988) Environ. Toxicol. Chem. 7, 859- 870
3. Harrison,R.O., Carlson,R.E., Shirkhan,H., Keimig,T. (1994) Organohalogen Compounds **19**, 167-172
4. Harrison,R.O., Carlson,R.E., Shirkhan,H. (1995) Organohalogen Compounds **23**, 187-19
5. Harrison,R.O., Carlson,R.E. (1997) Chemosphere **34**, 915-928
6. Harrison,R.O., Carlson,R.E. (1998) Organohalogen Compounds **35**, 43-46

7. Harrison,R.O., Carlson,R.E. (1999) Organohalogen Compounds **40**, 31-34
8. Sugawara,Y., Gee,S.J., Sanborn,J.R., Gilman,S.D., Hammock,B.D. (1998) Anal. Chem. **70**, 1092-1099
9. Anjo,T., Okuyama,M., Satoh,M., Kambegawa,A., Matsuki,Y. (2000) Organohalogen Compounds, **45**, 224-227
10. Kobayashi, N., Oiwa, H., Goto, J. (1998) J. Steroid Biochem. Molec. Biol. **64**, 171-177



Compound	R1	R2
[illegible]2	$NHCO(CH_2)_3COOH$	H
[illegible]3	$NHCO(CH_2)_4COOH$	H
[illegible]5	CH=CHCOOH	Cl
[illegible]2	H	$O(CH_2)_3COOH$

Figure 1. Structures of Dioxin Haptens