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Improvement in dioxin analysis of human blood and their concentrations in blood of Yusho patients

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KEYWORDS

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(PCDDs);
Yusho

Summary

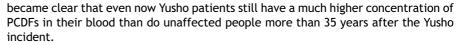
Background and objective: Over 35 years have passed since the Yusho incident. We have determined the concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-ortho-coplanar polychlorinated biphenyls (Co-PCBs) in blood samples collected from Yusho patients to establish new criteria for Yusho. Considering the fact that the concentrations of PCDDs, PCDFs and Co-PCBs in the blood samples of about 300 Yusho patients living in Japan were scheduled for measurement in 2002, it was desirable to develop more effective methods to speed up the pretreatment procedure for blood samples. In this study, we improved a method that allows many blood samples to be treated in a short period with high reproducibility in comparison with the previously described method. Using our method, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood collected from 279 Yusho patients in 2002 and 269 Yusho patients in 2003, and compared the results with those of 52 normal controls.

Methods: The extraction procedure of PCDDs, PCDFs and Co-PCBs from the blood samples was simplified. Concentrations of the PCDDs, PCDFs and Co-PCBs were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large volume injection system.

Results and conclusion: The lipid content and the concentration of each isomer of PCDDs, PCDFs and Co-PCBs in blood determined using the improved method were almost equal to those obtained by dioxin analysis organizations that used the conventional method to analyze the same blood samples. The improved method demonstrated high reproducibility based on experiments conducted using the same serum samples. These findings indicate that the improved method is essentially equivalent to the conventional method. From the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples of Yusho patients measured by the improved method, it

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1. Introduction

The Yusho poisoning accident, which affected over 1800 people, occurred in 1968 in western Japan, and was caused by the ingestion of rice bran oil used for cooking that contained the following contaminants: polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dib enzo-p-dioxins (PCDDs) [1]. This disease was soon found to be far more difficult to manage by conventional medical treatment than initially thought, primarily due to the fact that very high levels of these compounds were persistent in the tissue of Yusho patients. Over 35 years have passed since the Yusho incident, and almost all of the typical symptoms of the Yusho patients have improved. However, some patients are still afflicted with subjective symptoms. These patients still have a much higher concentration of PCDFs in their blood than do unaffected people [2-4]. Evidence that the environment is so extensively contaminated with these chlorinated hydrocarbons as to threaten the global ecosystem has become apparent. Therefore, the study of Yusho is significant not only for those affected with the disease but also for those who are known to be potentially contaminated with PCDDs, PCDFs and non-ortho-coplanar polychlorinated biphenyls (Co-PCBs).

Extensive studies with regard to Yusho have been conducting by the Study Group for Yusho [1]. We have determined the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples collected from Yusho patients to establish new criteria for Yusho [2-4]. Follow-up studies measuring the concentrations of PCDDs, PCDFs and Co-PCBs in the blood of Yusho patients are very important when considering the healthcare of these patients. With the conventional measuring method, 20-50 ml of blood is needed to exactly measure the concentrations of PCDDs, PCDFs and Co-PCBs [5]. However, because most of these patients are now over 60 years of age, collecting this amount of blood is restricted. These patients can safely supply only small volumes of blood for the measurement of PCDD, PCDF and Co-PCB concentrations. Therefore, to reduce the physical burden on patients, it was necessary to develop a highly sensitive analytic method that

could accurately evaluate PCDDs, PCDFs and Co-PCBs concentrations from small (5 g) blood samples.

In addition, given that the extraction procedure of PCDDs, PCDFs and Co-PCBs from the blood by the conventional method is very complicated and timeconsuming, it is not a suitable procedure for processing many samples. Recently, we developed an analytic method for measuring the concentrations of PCDDs, PCDFs and Co-PCBs in human blood samples as small as 5 g, and an efficient method for speeding up the pretreatment procedure for blood samples [6,7]. The method consists of three major steps: the extraction of lipid from human blood by an accelerated solvent extractor (ASE) system, a clean-up procedure at a scale one-quarter of that of the conventional method, and a sensitive determination method with high-resolution gas chromatography/ high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large volume (SCLV) injection system used in a large volume injection technique. By using HRGC/HRMS with a SCLV injection system, the sensitivity of the GC/MS was increased to 10 times that of the classical method. Therefore, it became possible for concentrations of PCDDs, PCDFs and Co-PCBs to be measured in a blood volume of 5 g.

Using this method, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples collected from 78 patients with Yusho living in Fukuoka Prefecture in 2001 [4]. Considering the fact that the concentrations of PCDDs, PCDFs and Co-PCBs in the blood samples of about 300 Yusho patients living in Japan were scheduled for measurement in 2002, it was desirable to develop more effective methods to speed up the pretreatment procedure for blood samples.

In this study, we developed a method that allows many blood samples to be treated in a short period with high reproducibility in comparison with the previously described method. We also examined efficient methods to reduce the background levels so that they do not affect the measurement of the PCDDs, PCDFs and Co-PCBs. Using these methods, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood collected from 279 Yusho patients in 2002 and 269 Yusho patients in 2003.

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