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Accumulation properties of polychlorinated biphenyl congeners in Yusho patients and prediction of their cytochrome P450-dependent metabolism by in silico analysis

Shusaku Hirakawa¹, Takashi Miyawaki¹, Tsuguhide Hori¹, Jumboku Kajiwara¹, Susumu Katsuki¹, Masashi Hirano², Yuka Yoshinouchi³, Hisato Iwata³, Chikage Mitoma⁴, and Masutaka Furue⁴

¹Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano, Dazaifu, Fukuoka 818-0135, Japan

²Kumamoto College, National Institute of Technology, 2627 Hirayamashin-Machi, Yatsushiro, Kumamoto 866-8501, Japan

³Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan

⁴Research and Clinical Center for Yusho and Dioxin, Kyusyu University Hospital, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

Abstract

In what has become known as the Yusho incident, thousands of people in western Japan were poisoned by the accidental ingestion of rice bran oil contaminated with polychlorinated biphenyls (PCBs) and various dioxins and dioxin-like compounds. In this study, we investigated the accumulation patterns of 69 PCB congeners in the blood of Yusho patients in comparison with those of non-exposed controls. The blood samples were collected at medical check-ups in 2004 and 2005. To compare the patterns of PCB congeners, we calculated the concentration ratio of each congener relative to the 2,2',4,4',5,5'-hexaCB (CB153) concentration. The concentration ratios of tetra- and penta-chlorinated congeners in the blood of Yusho patients were significantly lower than those of controls. To examine the cytochrome P450 (CYP)-dependent metabolic potential of the 2,3',4,4'-pentaCB (CB118), CB153, and 2,3,3',4,4'-hexaCB (CB156) congeners, we conducted PCB-CYP (CYP1A1, CYP1A2, CYP2A6, and CYP2B6) docking simulation by in silico analysis. The docking models showed that human CYP1A1, CYP2A6, and CYP2B6 isozymes have the potential to metabolize CB118 and CB153. On the other hand, it was inferred that CB156 is difficult to be metabolized by these four CYP isozymes. These results indicate that CYP1 and CYP2 isozymes may be involved in the characteristic accumulation patterns of PCB congeners in the blood of Yusho patients.

Keywords

Polychlorinated biphenyl; Metabolism; Cytochrome P450; Docking simulation

Introduction

In what has become known as the Yusho incident, thousands of people in western Japan were poisoned by the accidental ingestion of rice bran oil contaminated with polychlorinated biphenyls (PCBs) and various dioxins and dioxin-like compounds (Kuratsune et al. 1996). Since 1968, the year of the incident, the average concentration of PCBs in the blood of Yusho patients has shown a decreasing trend (Masuda et al. 2007). However, more than 40 years later, the concentration of total PCBs and polychlorinated dibenzofurans (PCDFs) in the blood of Yusho patients is still higher than that of controls (Kajiwara et al. 2015; Miyawaki et al. 2015). Furthermore, the composition of PCB congeners in the blood of Yusho patients is different from that of controls and is characterized by lower concentrations of 2,3',4,4'5-pentaCB (CB118) and higher concentrations of 2,3,3',4,4'5-hexaCB (CB156) (Masuda et al. 1974). This characteristic difference has been adopted as one of the criteria for the diagnosis of Yusho disease. However, overall accumulation properties including differences in other PCB congeners are still unclear.

In the metabolism of PCBs, hydroxylated PCBs (OH- PCBs) are well known as metabolites of PCBs formed by the cytochrome P450 (CYP) monooxygenase enzyme system (Grimm et al. 2015; Mills et al. 1985). Metabolic patterns of some PCB congeners have been reported in experimental animal studies (Haraguchi et al. 1997, 1998, 2005). PCBs will induce transactivation of CYP2 family isozymes mediated by constitutive androstane receptor (Gahrs et al. 2013; Sakai et al. 2006, 2009; Sueyoshi et al. 1999), and induced CYP2B metabolizes the PCB congener to hydroxylated PCBs (Safe et al. 1985). On the other hand, dioxins will induce transactivation of CYP1 family isozymes via aryl hydrocarbon receptor (Nebert et al. 2000; Okino and Whitlock 2000). It has been reported that CYP1 isozymes also metabolize PCB congeners (Koga et al. 1994; Schlezinger et al. 2000; Yamazaki et al. 2011). In recent studies, the CYP dependence of the metabolic potencies of PCB congeners was suggested by in silico docking simulation (Inui et al. 2014; Mise et al. 2016; Yoo et al. 2015).

In this study, we investigated the characteristics of the accumulation patterns of 69 PCB congeners in the blood of Yusho patients by comparing them to those of controls. In addition, to examine the CYP-dependent metabolic potential of PCB congeners, we conducted in silico docking simulations of PCB congeners (CB118 and CB153) with CYP isozymes (1A1, 1A2, 2A6, and 2B6).

Materials and methods

Blood sample collection and concentrations of PCB congeners

Medical check-ups for Yusho patients have been performed annually since the Yusho incident, in order to monitor the health status of the affected patients. Blood samples were collected at medical check-ups in 2004 (controls, $n = 127$) and 2005 (Yusho patients, $n = 237$), and informed consent was obtained from all participants (Table 1). Control samples unaffected by Yusho were collected in Fukuoka, Japan. The mean age of Yusho patients and controls was 67 and 68 years old, respectively. Concentrations of PCB congeners in the

blood of Yusho patients and controls were quantified by a combination of high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) and have already been reported (Hori et al. 2005; Todaka et al. 2008; Todaka et al. 2009). The average concentrations of the PCB congeners in the blood of Yusho patients and controls on a lipid-weight basis and the concentration ratios of congeners relative to the CB153 concentration are shown in Table 2.

In silico analyses

All in silico analyses were carried out using the Molecular Operating Environment (MOE) program (Chemical Computing Group, Montreal, Canada). To construct the 3D structure of heme-containing CYP proteins, the following templates of CYP1 and 2 isozymes were taken from the Protein Data Bank (<http://www.rcsb.org>): human CYP1A1 (PDB code: 4I8V), human CYP1A2 (PDB code: 2HI4), human CYP2A6 (PDB code: 1Z10), and human CYP2B6 (PDB code: 3QOA). Since the structural model of human CYP2B6 (PDB code: 3QOA) (Y226H/K262R) varies, we constructed an original structural model (H226Y/R262K) using MOE for the docking simulations. All crystallographic water molecules were deleted from the CYP structures. The 3D structures of human CYPs were optimized by a PFROSST force field after adding hydrogen atoms.

Molecular docking simulations were performed to simulate the binding of CB118 and CB153 congeners to human CYP proteins using ASEDock (Ryoka Systems Inc., Tokyo, Japan) following the method of Goto et al. (2008). Prior to the ASEDock analysis, structures of PCBs were constructed and their energies were minimized using Rebuild3D with the MMFF94x force field in the MOE. A total of 500 conformations for each PCB congener were generated by the LowMode MD method. The parameters used for the refinement step were as follows: a cutoff value of 4.5, RMS gradient of 10, and energy threshold of 500. The energy of the PCB-CYP complex was refined using PFROSST of MOE under limited conditions in which the backbones of amino acid residues were tethered and the side chains of amino acid residues were unconfined.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY). Wilcoxon signed-rank test was performed to compare the concentration ratios of PCB congeners containing each number of chlorine atoms between Yusho patients and controls. Tri-, nona-, and deca-chlorinated congeners were excluded from statistical analysis, because of the number of them were 1, 3, and 1, respectively (Table 2).

Results and discussion

Accumulation patterns of PCB congeners in Yusho patients and controls

We examined the accumulation patterns of 69 PCB congeners in the blood of Yusho patients and controls. For the comparison, we calculated the concentration ratio of each congener relative to CB153 (Table 2). The concentration level of CB153 was highest in both Yusho patients and controls, and the ratio of CB 118 and CB 156 to CB153 has been adopted as one of the criteria for the diagnosis of Yusho disease. Figure 1 shows the comparison of the

ratios to CB153 of Yusho patients and controls; results were log2-transformed. In this comparison, zero means the same level in both, and a plus or minus 1 value means a twofold difference. Same as previously explained (Masuda et al. 1974), the ratio of CB118 of Yusho patients was lower than that of controls. In addition, the ratio of CB156 of Yusho patients was higher than that of controls. In this study, we found that other congeners also showed differences, as well. Overall, the comparison indicated lower proportions of lower-chlorinated congeners in Yusho patients.

Next, we compiled the congeners for each number of chlorine atoms and compared the concentration ratios between Yusho patients and controls (Fig. 2a). As a result of Wilcoxon signed-rank test, the concentration ratios of tetra- and penta-chlorinated congeners in the blood of Yusho patients were significantly lower than those of controls. By comparison, those of hepta- and octa-chlorinated congeners in Yusho patients were significantly higher than those of controls. However, the concentration ratios of the contaminated rice bran oil and dietary fish (Hori et al. 2008; Miyawaki et al. 2015), which were considered the exposure sources, showed high rates of lower-chlorinated PCB congeners (Fig. 2b). Therefore, Yusho patients are considered to have been exposed to larger amounts of lower-chlorinated congeners than controls. From these results, we hypothesized that lower-chlorinated PCB congeners were more efficiently metabolized by metabolic enzymes induced in Yusho patients than were the higher-chlorinated congeners.

In silico analyses to evaluate CYP-dependent metabolic potential of PCB congeners

We examined the CYP-dependent metabolic potential of PCB congeners. Using the 3D structures of human CYPs (CYP1A1, CYP1A2, CYP2A6, and CYP2B6), PCB congeners were simulated in silico by the ASEDock program. In the present study, the docking positions for CB118, which showed a lower concentration in Yusho patients; CB153, which typically has the highest accumulation in the blood of humans; and CB156, which showed a higher concentration in Yusho patients, were compared among the human CYP proteins. For each PCB–CYP pair, we measured the distance between the Cl-unsubstituted carbon atom in the biphenyl ring of the PCBs and the heme iron in the CYPs. If the target (oxidation) site of the PCB congener is located within 5 or 6 Å of the heme iron, the substrate is supposed to be efficiently metabolized by CYP (de Graaf et al. 2006; Sykes et al. 2008; Vasanthanathan et al. 2009). In this study, we investigated whether the target site is within 5 Å of heme iron of CYP. As results of docking simulation, 6, 8, 7, and 9 docking poses of CB118 were confirmed in CYP1A1, CYP1A2, CYP2A6, and CYP2B6, respectively. The docking poses of CB118 nearest to the heme iron in human CYP isozymes are shown in Fig. 3. On the basis of the distance in silico estimated for each human CYP (4.75 Å for CYP1A1, 5.41 Å for CYP1A2, 3.86 Å for CYP2A6, and 4.83 Å for CYP2B6), it was inferred that CYP1A1, CYP2A6, and CYP2B6 may be efficient catalysts for CB118. The average value of the shortest distances of target site in all the docking poses were 6.90 Å for CYP1A1, 6.18 Å for CYP1A2, 4.80 Å for CYP2A6, and 5.29 Å for CYP2B6. Therefore, in particular, human CYP2A6 was presumed to have a substrate binding pocket for efficiently metabolizing CB118. Next, we checked the position of the target site of PCB structure. The target site by CYP2A6 and CYP2B6 was the 3-position of the PCB structure (Fig. 3c, d). This position will be hydroxylated and lead to the production of 3-OH-CB118 and 4-OH-CB107 (Grimm

et al. 2015). 4-OH-CB107 is one of the frequently detected OH-PCB congeners in human blood samples and has also been found in the blood of Yusho patients (Linderholm et al. 2007; Tobiishi et al. 2013). Mise et al. (2016) reported that human CYP2B6 and rat CYP2B1 metabolized CB118 to 3-OH-CB118, and the docking models revealed short distances between the 3- position of CB118 and these CYP2B isozymes. In this study, the target site of CB118 by CYP1A1 was the 2'-position of the PCB structure (Fig. 3a). An in vitro study reported that rat CYP1A1 metabolized CB118 to 4-OH-CB107 whereas human CYP1A1 was not involved in the metabolism of CB118 to 4-OH-CB107 (Mise et al. 2016). On the other hand, a review of the metabolism of PCBs reported that 2',4-diOH- CB107 was identified in human plasma (Grimm et al. 2015). This suggests that there may be a hydroxylation pathway at the 2'-position of a PCB structure by human CYP1A1 isozyme.

The docking poses of CB153 nearest to the heme iron in human CYP isozymes are shown in Fig. 4. Docking models of CYP1A1, CYP2A6, and CYP2B6 indicated that the target site of CB153 could be positioned within 5 Å of the heme iron. This result suggests that these CYP isozymes are effective catalysts for CB153. The average value of the shortest distances of target site was 5.73 Å for CYP1A1 in 7 docking poses, 5.60 Å for CYP1A2 in 8 docking poses, 4.54 Å for CYP2A6 in 9 docking poses, and 5.22 Å for CYP2B6 in 6 docking poses. As with CB118, it was inferred that especially human CYP2A6 have a substrate binding pocket for efficiently metabolizing CB153. In the blood of Yusho patients, the concentration levels of CB153 slowly decreased in the 38 years after the incident (Masuda et al. 2007). From the results of in silico docking simulation, we speculated that the target site nearest the heme iron of CYP1A1 was the 6- position of the PCB structure (Fig. 4a), whereas the target site nearest that of CYP2A6 and CYP2B6 was the 3-position of PCB structure (Fig. 4c, d). The hydroxylated CB153 at the 6- position was not detected in the blood of Yusho patients. However, 3-OH-CB153 and 4-OH-CB146 are generated by the hydroxylation of the 3-position of CB153. These OH-PCB congeners are commonly detected in human blood (Grimm et al. 2015) and have also been detected in the blood of Yusho patients (Linderholm et al. 2007; Tobiishi et al. 2013). This suggests that CYP2A6 and CYP2B6 may be responsible for the hydroxylation of the 3-position of CB153.

In addition, we investigated the docking poses of CB156 nearest to the heme iron in human CYP isozymes (Fig. 5). Docking models of CYP1A1 and CYP2A6 indicated that the target site of CB156 could be positioned within 5 Å of the heme iron, 2'-position and 6-position of PCB structure, respectively. However, the hydroxylated CB156 at these positions have been identified neither in Yusho patients nor in the review of PCB metabolism. Further, the average value of the shortest distances of target site was more than 5 Å in all four CYP isozymes (6.41 Å for CYP1A1 in 8 docking poses, 5.53 Å for CYP1A2 in 8 docking poses, 5.06 Å for CYP2A6 in 6 docking poses, and 5.95 Å for CYP2B6 in 6 docking poses). From these results, it was inferred that CB156 is difficult to be metabolized by these four CYP isozymes.

The present study suggests that an in silico docking simulation is useful for the prediction of CYP-dependent metabolism of PCB congeners, since the results of docking simulation were consistent with the data of in vitro and measurement studies of hydroxylated PCB congeners. We have shown that human CYP1A1, CYP2A6, and CYP2B6 have the potential

to metabolize PCB congeners. In addition, CYP1A1 and CYP2A6 are induced by an aryl hydrocarbon receptor-mediated pathway (Arpiainen et al. 2005; Murtomaa-Hautala et al. 2012; Nebert et al. 2000; Okino and Whitlock 2000). Because the concentration of PCDFs in the blood of Yusho patients is still higher than that of controls, these isozymes may be involved in the characteristic accumulation patterns of PCB congeners in the blood of Yusho patients.

Conclusions

The present study showed that the contents of tetra- and penta-chlorinated congeners in the blood of Yusho patients were significantly lower than those of controls. In addition, examination of the CYP-dependent metabolic potential of CB118, CB153, and CB156 congeners by *in silico* docking simulation indicated that CYP1A1, CYP2A6, and CYP2B6 have the potential to metabolize CB118 and CB153, and these CYPs may affect the accumulation properties of PCBs in Yusho patients. On the other hand, it was inferred that CB156 is difficult to be metabolized by CYP1A1, CYP1A2, CYP2A6, and CYP2B6. In this study, we conducted docking simulations of three PCB congeners with a limited number of CYP isozymes. Further study is necessary to develop a comprehensive picture of CYP-dependent PCB metabolism in humans by docking simulations between other PCB congeners detected in human blood and a variety of human CYP isozymes.

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Fig. 1.

Comparison of the concentration ratios of PCB congeners relative to CB153 in Yusho patients and controls. The concentration ratios of Yusho patients and controls were log₂-transformed. The plots indicate each congener showed IUPAC number

Fig. 2.

Comparison of the concentration ratios of PCB congeners relative to CB153. a Yusho patients and controls. b Rice bran oil and dietary fish. PCB congeners were compiled for each number of chlorine atoms. Concentration data were cited from Hori et al. 2008, Miyawaki et al. 2015, and Todaka et al. 2009. * $p < 0.05$; ** $p < 0.01$ (Wilcoxon signed-rank test)

Fig. 3. Comparison of docking poses of CB118 in human CYP isozymes. a CYP1A1. b CYP1A2. c CYP2A6. d CYP2B6. The shortest distance (Å) between the Cl-unsubstituted carbon atom of CB118 and the heme Fe is shown by the *blue line*

Fig. 4.
Comparison of docking poses of CB153 in human CYP isozymes. a CYP1A1. b CYP1A2. c CYP2A6. d CYP2B6. The shortest distance (Å) between the Cl-unsubstituted carbon atom of CB153 and the heme Fe is shown by the *blue line*

Fig. 5. Comparison of docking poses of CB156 in human CYP isozymes. a CYP1A1. b CYP1A2. c CYP2A6. d CYP2B6. The shortest distance (Å) between the Cl-unsubstituted carbon atom of CB156 and the heme Fe is shown by the *blue line*

Table 1

Sample data of Yusho patients and controls

	Yusho patients (collected in 2005)			Controls (collected in 2004)		
	Male	Female	Total	Male	Female	Total
Number	<i>n</i> = 107	<i>n</i> = 130	<i>n</i> = 237	<i>n</i> = 51	<i>n</i> = 76	<i>n</i> = 127
Mean age	68	66	67	68	68	68
Age breadth	36–88	37–91	36–91	60–79	60–86	60–86

Concentrations and concentration ratios of PCB congeners in the blood of Yusho patients and controls cited from Todaka et al. (2009)

IUPAC no.	Concentration (pg/g lipid wt.)			Concentration ratios of each congener/CB153		
	Yusho patients Mean (n = 237, 2005)	Controls Mean (n = 127, 2004)	Yusho patients	Yusho patients	Controls	Controls
IUPAC no.	Concentration (pg/g lipid wt.)			Concentration ratios of each congener/CB153		
	Yusho patients Mean (n = 237, 2005)	Controls Mean (n = 127, 2004)	Yusho patients	Yusho patients	Controls	Controls
244'-TrCB(no. 28)	1300	2500	0.010	0.028	0.0036	0.0036
22'35'-TeCB(no. 44)	310	450	0.0023	0.0050	0.16	0.15
22'44'-TeCB(no. 47)	430	600	0.0032	0.0067	0.0045	0.0053
22'45'-TeCB(no. 49)	230	300	0.0017	0.0033	0.0093	0.013
22'55'-TeCB(no. 52)	970	1200	0.0072	0.014	1	1
233'4'-2344'-TeCBs(no. 56/60)	480	880	0.0036	0.0098	0.23	0.088
234'5'-TeCB(no. 63)	84	140	0.00062	0.0016	0.062	0.022
23'44'-TeCB(no. 66)	1500	2300	0.011	0.026	0.2	0.21
23'4'5'-TeCB(no. 70)	190	250	0.0014	0.0028	0.029	0.040
23'4'6'-TeCB(no. 71)	87	190	0.00064	0.0021	0.29	0.19
244'5'-TeCB(no. 74)	9800	19,000	0.073	0.21	0.044	0.033
22'344'-PeCB(no. 85)	140	210	0.0010	0.0024	0.063	0.064
22'345'-PeCB(no. 87)	640	690	0.0048	0.0076	0.070	0.069
22'355'-PeCB(no. 92)	600	860	0.0044	0.0095	0.0016	0.0023
22'35'6'-PeCB(no. 95)	660	830	0.0049	0.0092	0.82	0.66
22'44'5'-PeCB(no. 99)	17,000	12,000	0.13	0.13	0.0023	0.00078
22'455'-PeCB(no. 101)	1600	1800	0.012	0.021	0.32	0.31
233'44'-PeCB(no. 105)	3400	5000	0.025	0.056	0.077	0.068
233'4'5'-PeCB(no. 107)	690	980	0.0051	0.010	0.033	0.011
233'4'6'-PeCB(no. 110)	360	410	0.0027	0.0045	0.013	0.0085
2344'5'-PeCB(no. 114)	1800	1600	0.014	0.018	0.14	0.095
234'56'-PeCB(no. 117)	1400	920	0.010	0.010	0.032	0.020
23'44'5'-PeCB(no. 118)	16,000	24,000	0.12	0.27	0.14	0.087

IUPAC no.	Concentration (pg/g lipid wt.)		Concentration ratios of each congener/CB153		IUPAC no.	Concentration (pg/g lipid wt.)		Concentration ratios of each congener/CB153	
	Yusho patients Mean (n = 237, 2005)	Controls Mean (n = 127, 2004)	Yusho patients	Controls		Yusho patients Mean (n = 237, 2005)	Controls Mean (n = 127, 2004)	Yusho patients	Controls
2'344'5'-PeCB(no. 123)	270	460	0.0020	0.0051	22'33'45'6-/22'33'455'6'-OcCB(no. 198/199)	25,000	10,000	0.19	0.11
22'33'44'-HxCB(no. 128)	860	870	0.0064	0.0097	22'33'45'66'-/22'344'566'-OcCB(no. 201/204)	660	640	0.0049	0.0072
22'33'45'-HxCB(no. 130)	4400	2600	0.033	0.029	22'33'55'66'-OcCB(no. 202)	4500	2800	0.033	0.031
22'33'46'-HxCB(no. 132)	180	280	0.0013	0.0031	233'44'55'6'-OcCB(no. 205)	870	300	0.0064	0.0034
22'33'56'-HxCB(no. 134)	25	27	0.00018	0.00030	22'33'44'55'6'-NoCB(no. 206)	3000	1900	0.022	0.021
22'33'56'-HxCB(no. 135)	470	470	0.0034	0.0052	22'33'44'566'-NoCB(no. 207)	410	330	0.0030	0.0037
22'344'5'-HxCB(no. 137)	6000	2900	0.044	0.033	22'33'455'66'-NoCB(no. 208)	940	770	0.0070	0.0086
22'344'5'-HxCB(no. 138)	66,000	40,000	0.49	0.45	22'33'44'55'66'-DeCB(no. 209)	1100	1300	0.0085	0.015
22'344'6-/22'34'5'6-HxCB(no. 139/149)	740	820	0.0055	0.0091	Total PCBs	680,000	430,000		