IN SILICO PREDICTION OF THE METABOLISM OF PCB CONGENERS BY CYTOCHROME P450 ISOZYMES IN YUSHO PATIENTS

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Introduction

In the Yusho incident of 1968, 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 [1]. Although the concentrations of total PCBs and polychlorinated dibenzofurans (PCDFs) in the blood of Yusho patients have shown a decreasing trend during a half century [2], they are still higher than those of controls [3, 4]. In addition, the composition of PCB congeners in the blood of Yusho patients 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) [5]. This compositional difference has been adopted as one of the criteria for the diagnosis of Yusho disease. Our previous study indicated that the concentration ratios of tetra- and penta-chlorinated congeners to 2,2',4,4',5,5'-hexaCB (CB153) are significantly lower in Yusho patients than in controls, whereas the concentration ratios of hepta- and octa-chlorinated congeners to CB153 are significantly higher in Yusho patients [6]. Thus, we hypothesized that tetra- and penta-chlorinated congeners are metabolized by cytochrome P450 (CYP) isozymes induced by PCBs and PCDFs in Yusho patients.

Hydroxylated PCBs (OH-PCBs) are well known as metabolites of PCBs formed by CYP enzymes. Exposure to non-dioxin-like PCBs induces the transcription of CYP2 family isozymes and enhances CYP2B-mediated metabolism of PCB congeners to OH-PCBs [7]. CYP1 isozymes, which are induced by dioxin-like congeners, also metabolize PCBs in a congener-specific manner [8]. In recent studies, the isozyme specificity of CYP metabolic potencies for different PCB congeners was suggested by *in silico* docking simulations [9-11]. We also examined the CYP1A1-, CYP1A2-, CYP2A6- and CYP2B6-specific metabolic potencies for CB118, CB153, and CB156 congeners by *in silico* docking simulations, and found that CYP1A1, CYP2A6, and CYP2B6 have the potency to metabolize CB118, whereas CB156 is unlikely to be metabolized by any of these four CYP isozymes [6]. Since these results of the docking simulations were consistent with findings of previous reports, such as an *in vitro* experiment and measurement of PCB congeners [4, 10], *in silico* docking simulation may be a useful method for predicting the isozyme specificity of CYP metabolism of PCB congeners.

To examine the CYP isozyme-dependent metabolic potential of PCB congeners, we conducted *in silico* docking simulations on 69 PCB congeners and seven CYP isozymes and investigated which CYP isozymes could metabolize which PCB congeners, and if metabolized, which OH-PCB congeners were produced.

Materials and methods

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 [12]. In this study, we performed docking simulation using CYP isozymes on 69 PCB congeners detected in the blood of Yusho patients.

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 CYP isozymes were taken from the Protein Data Bank (http://www.rcsb.org): human CYP1A1 (PDB code: 4I8V), human CYP1A2 (PDB code: 2HI4), human CYP1B1 (PDB code: 3PM0), human CYP2A6 (PDB code: 1Z10), human CYP2B6 (PDB code: 3QOA), human CYP2C9 (PDB code: 1OG5), and human CYP3A4 (PDB code: 1TQN). 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 hydrogen atoms were added. Molecular docking simulations were performed to simulate the binding of 69 PCB congeners to human CYP proteins using ASEDock (MOLSIS Inc., Tokyo, Japan) following the method of Goto et al. (2008) [13]. Prior to the ASEDock analysis, PCB structures 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: cutoff value, 4.5; RMS gradient, 10; and energy threshold, 500. The energy of the PCB–CYP complex was refined using PFROSST in MOE under limited

conditions in which the backbones of amino acid residues were tethered and the side chains of amino acid residues were unconfined.
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 was located within 5 or 6 Å of the heme iron, the substrate was considered to have been efficiently metabolized by CYP [14-16]. Thus, as one of the evaluation items of PCB metabolism, we investigated whether the target site was within 5 Å of the heme iron of CYP. As the results of the docking simulation, several docking poses were confirmed. Therefore, we calculated the average value of the distances of the target site in all docking poses, and investigated whether within it was 5 Å. In addition, we checked the position of the target site of the PCB structure, to confirm whether the expected OH-PCB was present, based on previously reported findings in the review by Grimm et al. (2015) [17].

Results and discussion

The accumulation patterns of 69 PCB congeners in the blood of Yusho patients compared to controls was previously reported in Hirakawa et al. (2017) and is shown in the upper part of Fig. 1 [18]. The concentration ratio of each congener relative to CB153 and the log2-transfomed ratios of Yusho patients to controls were calculated. In this comparison, zero means the same level in both, and a plus or minus 1 value means a 2-fold difference. As previously reported in Hirakawa et al. (2018) [6], tetra- and penta-chlorinated congeners in the blood of Yusho patients were significantly lower than those of controls, whereas hepta- and octa-chlorinated congeners in Yusho patients were significantly higher than those of controls. However, the analysis also indicated differences in the accumulation patterns in the same-number-chlorination PCB congeners. Presumably, the substitution positions of the chlorines in each PCB congener and the target site of the CYP isozyme are responsible for these differences. Thus, we conducted *in silico* docking simulations between 69 PCB congeners and seven CYP isozymes; the resulting list of metabolic potentials for these PCB congeners by CYP isozymes are shown in the lower part of Fig. 1. Light orange means that the shortest distance of the target site in all docking poses is within 5 Å from the heme iron of CYP. Dark orange means that the average value of the distances of the target site in all docking poses is within 5 Å. Red dots indicate what was inferred as the parent PCB congener of OH-PCB predicted from the target site in the review of Grimm et al. (2015) [17]. The docking models showed that human CYP2A6 and CYP2B6 had metabolic potential for most of the PCB congeners, followed by CYP1A1, whereas CYP1A2 and CYP1B1 have metabolic potential for only some PCB congeners. On the other hand, CYP2C9 and CYP3A4 were shown to be hardly involved in PCB metabolism.

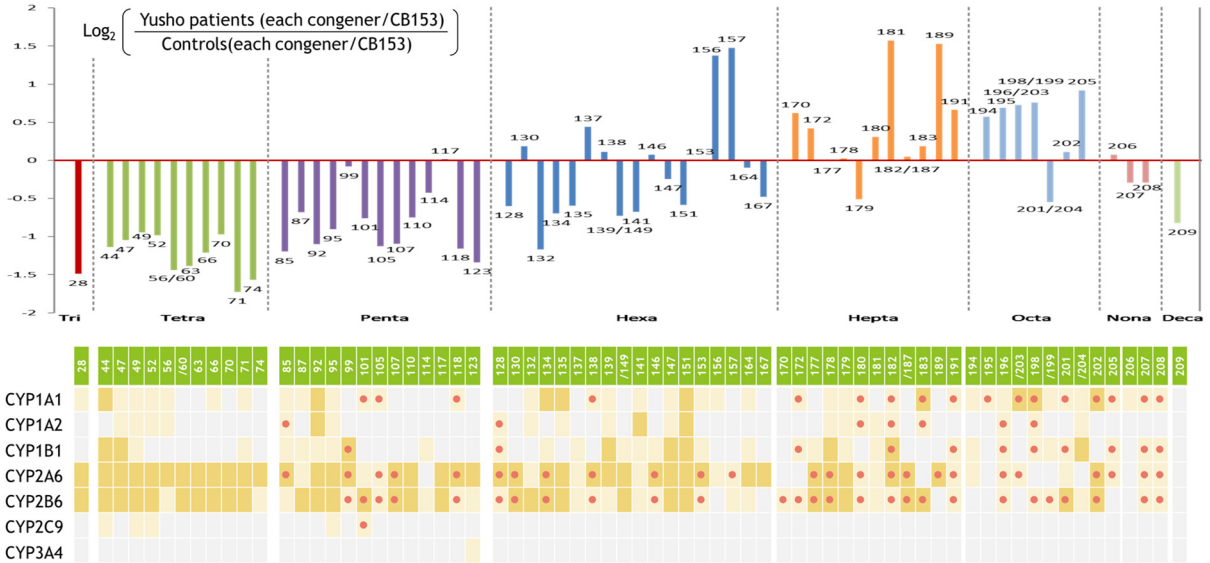

Fig. 1 The accumulation patterns of 69 PCB congeners in the blood of Yusho patients compared to controls (in upper part) [18], and the list of CYP-dependent metabolic potential of these PCB congeners predicted by *in silico* docking simulation (in lower part).
Light orange: the shortest distance of the target site in all docking poses is within 5 Å from the heme iron of CYP.
Dark orange: the average value of the distances of the target site in all docking poses is within 5 Å.
Red dots: what was inferred as the parent PCB congener of OH-PCB predicted from the target site in the review of Grimm et al. (2015) [17].

Focusing on PCB congeners, although tri- and tetra-chlorinated congener metabolisms by CYP isozymes using docking models were predicted, the inferred target sites of tri- and tetra-chlorinated congeners by simulation were not reported in the review of Grimm et al. (2015) [17]. Since this review summarized OH-PCBs in blood, it is possible that OH-PCBs that are difficult to transfer into the blood or are rapidly metabolized were not detected. In Yusho patients, low-chlorinated PCB congeners may be preferentially metabolized as mentioned above, it is likely that the CYP isozymes indicated in Fig. 1 as having high metabolic potentials are involved. In addition, the docking models showed the possibility of CYP-mediated metabolism even for highly-chlorinated PCB congeners, and the inferred target sites by simulation were consistent with the parent PCB congeners of OH-PCBs reported in Grimm et al. (2015) [17]. Therefore, even persistent highly-chlorinated PCB congeners may be metabolized by the CYP isozymes indicated with red dots in Fig. 1.

Previously, studies of the metabolic pathways of PCBs have been reported, based on *in vivo* investigations of the OH-PCBs generated in experimental animals exposed to specific PCBs [19-21]. However, although this experimental method is effective for estimating the parent PCB congener that produces the hydroxide, it is difficult to determine which CYP is involved. In this study, we used the results of docking simulation to infer the parent PCB congeners and CYP isozymes involved in the hydroxide formation pathway for 4-OH-CB187, 4-OH-CB146 and 4-OH-CB107, which are abundant in the blood of human. Grimm et al. (2015) predicted the parent PCB congeners of 4-OH-CB187 to be 2,2',3,4,4',5',6-heptaCB (CB183) and 2,2',3,4',5,5',6-heptaCB (CB187), those of 4-OH-CB146 to be 2,2',3,4,4',5'-hexaCB (CB138), 2,2',3,4',5,5'-hexaCB (CB146) and CB153, and those of 4-OH-CB107 to be 2,3,3',4,4'-pentaCB (CB105), 2,3,3',4',5-pentaCB (CB107) and CB118 [17]. Fig. 2 shows these OH-PCBs and their parent PCB congeners, along with the CYP isozymes whose involvement in the metabolic pathway was inferred from docking simulation. It was speculated that CYP2B6 was involved in the pathway that generated 4-OH-CB187 from CB187 and CB183. Likewise, it was speculated that CYP2A6 and CYP2B6 were involved in the pathways that generated 4-OH-CB146 from CB138, CB146 and CB153, and that CYP1A1 was also involved in the pathway that generated the 4-OH-CB146 from CB138. In the case of 4-OH-CB107, it was speculated that CYP2A6 and CYP2B6 were involved in the pathway from CB107 and CB118, and that CYP2B6 was involved in the pathway from CB105. Thus, it was possible to estimate the CYP isozymes involved in the metabolic pathway of OH-PCBs from the parent congener by analyzing the target site using docking simulation.

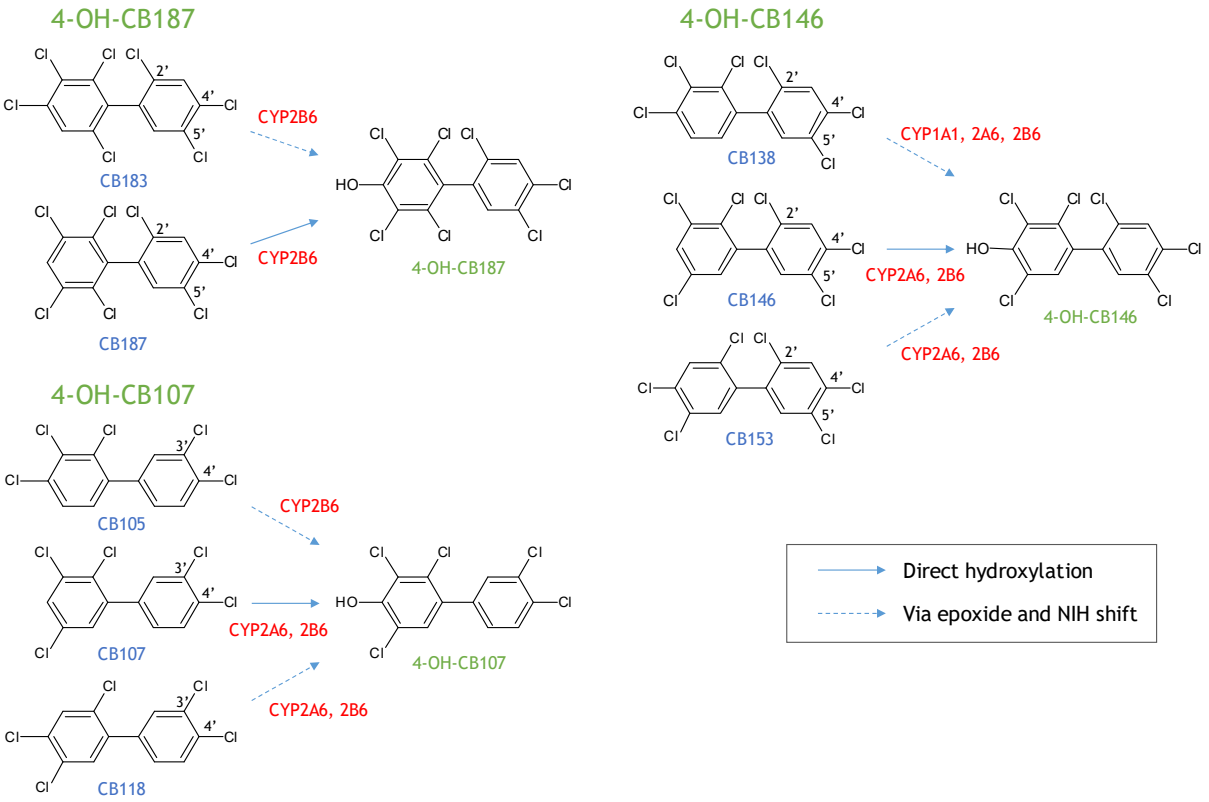

Fig. 2 The expected parent compounds of 4-OH-CB187, 4-OH-CB146 and 4-OH-CB107 [17], and predicted CYP isozymes involved in metabolic pathways by *in silico* analysis.

The present study suggests that it is possible to infer the generation of OH-PCBs by analyzing target sites using PCB-CYP docking simulation. The effectiveness of the docking simulation can be evaluated by verifying whether the predicted metabolic pathway actually occurs using *in vitro* experiments or the like. In the future, it may be possible to understand what kind of CYP isozymes are affected by rice oil exposure by investigating differences in the accumulation patterns of OH-PCBs in Yusho patients and controls.

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