

### Short Communication

## Aryl Hydrocarbon Hydroxylase Represents CYP1B1, and not CYP1A1, in Human Freshly Isolated White Cells: Trimodal Distribution of Japanese Population According to Induction of CYP1B1 mRNA by Environmental Dioxins<sup>1</sup>

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#### Abstract

The expression level of mRNAs for cytochrome P450 (CYP) 1A1 and 1B1 in freshly prepared white cells from 72 subjects exposed to dioxins at waste incinerators was investigated. The amounts of CYP1B1 mRNA ranged from 0.16 to 671 molecules/10<sup>7</sup> molecules of 18S rRNA, whereas the amounts of CYP1A1 mRNA were <6 molecules/10 ng total RNA, indicating that CYP1A1 was not induced to a detectable level by environmentally exposed dioxins. The inducibility of CYP1B1 mRNA in leukocytes, defined as the ratio of CYP1B1 mRNA to the plasma concentration of dioxins, varied among the subjects. It was found that the subjects showed trimodal distribution according to inducibility: 39 (54.2%), 25 (34.7%), and 8 (11.1%) of 72 subjects were judged as poor, intermediate, and high responders to environmental dioxins, respectively. The amounts of CYP1B1 mRNA in leukocytes of the intermediate and high responders were highly correlated with the plasma concentrations of dioxins ( $P < 0.05$  and  $<0.01$ ). These results suggest that CYP1B1 with polymorphic inducibility by dioxins is involved in aromatic hydrocarbon hydroxylase activities in human lymphocytes.

#### Introduction

Carcinogenic PAHs<sup>3</sup> are metabolically activated by enzymes to generate reactive intermediates capable of binding to DNA to

cause the mutation of cancer-related genes (1, 2). Thus, the capacity of enzymes responsible for the activation of the carcinogenic PAHs has been regarded as one of the factors affecting the risk to chemical carcinogenesis. Kellermann *et al.* (3) first reported that the capacity of individuals to induce AHH in lymphocytes in response to PAH was apparently associated with the individual differences in the risk of bronchogenic carcinoma, suggesting that the amount of AHH was one of the key determinants of the cancer risk. After this result, Guirgis *et al.* (4) and Trell *et al.* (5) reported that AHH activity in individuals correlated well with the risk of lung cancer and laryngeal carcinoma. The original assay of Kellermann *et al.* (3) was to measure PAH-inducible AHH activity in mitogen-activated lymphoblasts cultured for 96 h.

CYP1A1 had been thought to be a sole enzyme acting as AHH until the role of CYP1B1 in the metabolic activation of PAHs was discovered (6, 7). The role of CYP1B1 in the metabolic activation of chemical carcinogens was found during the course of studies on the responsiveness to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (8). Thus, it seemed interesting to determine whether the AHH activity in the lymphoblast cultures prepared by the method of Kellermann *et al.* (3) was attributable to CYP1A1 or CYP1B1.

We initiated this study to determine whether either one or both mRNAs for CYP1A1 and CYP1B1 were induced in human leukocytes in response to environmental dioxins in Japanese incinerator workers. We found that CYP1B1 mRNA, but not CYP1A1 mRNA, was detectable in essentially all subjects and that there were individual variations in the responsiveness to environmental dioxins to induce CYP1B1 mRNA.

#### Materials and Methods

**Human Blood Samples.** Blood samples used in this study were taken from Japanese subjects who were occupationally exposed to dioxins at waste incinerators. The concentrations of dioxins (pg TEQ/g lipid), which were defined as the sum of polychlorinated dibenzo-*p*-dioxins normalized with a WHO toxic equivalent factor, were determined by gas chromatography high-resolution mass spectrometry as described previously (9). This study was approved by the ethics committee of Hokkaido University.

**First-strand cDNA Synthesis.** Total RNAs in human leukocytes were isolated by a TRizol LS reagent system (Invitrogen, Carlsbad, CA). Nothing was done to separate the different white cell types. First-strand cDNA was then synthesized. Reverse transcriptase reaction was performed under the following conditions: a reaction mixture contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM DTT, 0.5 mM each deoxynucleotide triphosphate, 10 ng/ $\mu$ l total RNA, 4.5 ng/ $\mu$ l random primer pd(N)<sub>6</sub> (Promega, Madison, WI), 2 units/ $\mu$ l a

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<sup>3</sup> The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; AHH, aromatic hydrocarbon hydroxylase; CYP, cytochrome P450; TEQ, toxic equivalents to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

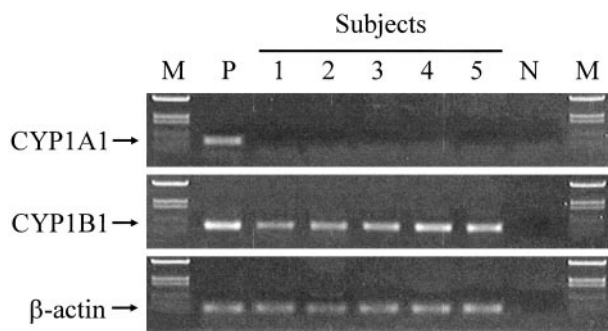


Fig. 1. Expression of CYP1A1 and CYP1B1 mRNA in total RNA prepared from 5 subjects who might be highly disclosed to dioxins. An amplified fragment size for CYP1A1, CYP1B1, or  $\beta$ -actin is 139, 139, or 78 bp, respectively. M, DNA size marker; N, none template control; P, positive control.

Maloney murine leukemia virus reverse transcriptase (Promega), and 0.4 units/ $\mu$ l RNase inhibitor (Toyobo, Osaka, Japan). The mixtures were treated at 25°C for 10 min and then 37°C for 60 min.

**PCR Condition.** Primers used for the amplification of cDNAs for CYP1A1 and CYP1B1 were CYP1A1-RTF, 5'-ccttcacatctggagacctcc-3'; CYP1A1-RTR, 5'-atggttgatctgccactgtgtt-3'; CYP1B1-RTF, 5'-agaacgtaccggccaactac-3'; and CYP1B1-RTR, 5'-cacgacctgatccaattctg-3'. A quantitative PCR to determine the amount of CYP1B1 mRNA was performed in a real-time PCR machine, LightCycler (Roche Diagnostics, Mannheim, Germany), using a LightCycler-FastStart DNA Master SYBR Green I (Roche Diagnostics) according to the manufacturer's instructions. PCR amplifications were performed in 40 cycles with melting at 95°C for 15 s, annealing at 60°C for 5 s, and extension at 72°C for 7 s. The amounts of 18S rRNA used as an internal standard were determined by a TaqMan Ribosomal RNA Control Reagents VIC Probe (Applied Biosystems, Foster City, CA). PCR conditions for amplification of  $\beta$ -actin mRNA were as described previously (10).

## Results

**Expression of mRNAs for CYP1A1 and CYP1B1.** The expression of mRNAs for CYP1A1 and CYP1B1 in leukocytes from 72 subjects was quantified with reverse transcription-PCR. Although the expression of CYP1B1 mRNA and  $\beta$ -actin mRNA was detectable in all subjects, the expression of CYP1A1 mRNA could not be seen under present experimental conditions (Fig. 1). We could not detect the expression of CYP1A1 mRNA in all subjects with either ABI 7700 (Applied Biosystems) or TaqMan probe (Applied Biosystems) in addition to the real-time PCR, with a detection limit of CYP1A1 mRNA as low as 6 molecules/10 ng total RNA (data not shown).

**Inducibility of the CYP1B1 Gene.** The expression level of CYP1B1 mRNA in human leukocytes was quantified with the quantitative reverse transcription-PCR. The amounts of CYP1B1 mRNA are shown as the copy number of CYP1B1 mRNA/ $10^7$  molecules of 18S rRNA. As shown in Fig. 2A, the amounts of CYP1B1 mRNA in total RNAs prepared from 72 human leukocytes ranged from 0.18 to 671 molecules/ $10^7$  molecules of 18S rRNA. The ratio of the amounts of CYP1B1 mRNA to the concentration of dioxins in plasma was defined as the inducibility of CYP1B1 mRNA. The ratio varied over three orders of magnitude. The probit plot analysis for the distribu-

tion of the inducibility of CYP1B1 mRNA is shown in Fig. 2B. The results indicate the trimodal distribution of subjects according to the inducibility of CYP1B1 mRNA. Thus, low, intermediate, and high inducibility groups consisted of 39 (54.2%), 25 (34.7%), and 8 (11.1%) subjects, respectively.

**Correlation between the Amounts of CYP1B1 mRNA and Concentrations of Dioxins.** To determine whether a correlation was seen between the amounts of CYP1B1 mRNA and concentrations of dioxins, least squared regression analysis was performed in high, intermediate, and low inducibility subjects, respectively (Fig. 3). The amounts of CYP1B1 mRNA significantly correlated with the concentrations of dioxins in the high inducibility group ( $n = 8$ ,  $r = 0.86$ ,  $P < 0.01$ ). A low but significant correlation coefficient was also observed in the intermediate inducibility group ( $n = 25$ ,  $r = 0.41$ ,  $P < 0.05$ ). There was no correlation in the low group.

## Discussion

The CYP1A1 and CYP1B1 genes are members of aromatic hydrocarbon receptor battery genes. The expression of these genes, therefore, is thought to be induced concomitantly through the activation of aromatic hydrocarbon receptor. In agreement with this concept, both CYP1A1 and CYP1B1 mRNAs were induced by the treatment of human blood monocytes with dioxins or PAHs *in vitro* (11, 12). The subjects whose leukocytes were analyzed in this study were workers occupationally exposed to dioxins at waste incinerators (9). Thus, we expected that both CYP1A1 and CYP1B1 mRNAs might be induced by environmental exposure to dioxins. However, we found that the expression of only CYP1B1 mRNA was induced, whereas the expression of CYP1A1 mRNA was not induced. The reason accounting for the fact that only CYP1B1 mRNA was induced is not known at present.

After the first report on the significant association between AHH inducibility in human lymphocytes and the bronchogenic cancer risk (3), many papers have appeared to argue that the apparent association was not seen between the AHH inducibility and susceptibility to lung cancer or other cancers (13–17). The reason for this apparent discrepancy has been partly accounted for by the difficulty of the measurement and the seasonal variation of the AHH activity in human lymphocytes (18, 19). It has long been believed that CYP1A1 is the major isoform involved in AHH activities in human lymphocytes (7). Recent reports have indicated that both human CYP1A1 and CYP1B1 are involved in the AHH activities (7, 8, 20). In addition, CYP1B1 is reported to exist as the major CYP isoform in human blood monocytes (11). Although there is a fundamental difference between AHH activities in the cultured mitogen-activated lymphoblasts reported previously (3–5) and the mRNA levels in the freshly isolated leukocytes in this study, together with the data presented herein, it may be reasonable to assume that CYP1B1 rather than CYP1A1 is responsible for the AHH activity in human lymphocytes *in vivo*. Additional experiments still need to be done in this context.

A trimodal distribution of subjects according to the inducibility of AHH activity in human lymphocytes was reported in a previous report, which was apparently associated with the cancer risk (3). In agreement with the previous report, we found a similar trimodal distribution in the inducibility of CYP1B1 mRNA based on the calculated ratio of the amounts of CYP1B1 mRNA to the concentrations of dioxins in plasma (Fig. 2B), lending further support to the idea that CYP1B1 rather than CYP1A1 is likely to be a major CYP isoform involved in AHH activity in human lymphocytes. The polymorphic inducibility

Fig. 2. A, the amount of CYP1B1 mRNA in human blood. The amount of CYP1B1 mRNA represents copy number of CYP1B1 mRNA/ $10^7$  copies of 18S rRNA (molecules/ $10^7$  molecules of 18S rRNA). B, probit plot for the distribution of CYP1B1 mRNA inducibility in human leukocytes. The ratio between the amount of CYP1B1 mRNA (molecules/ $10^7$  molecules of 18S rRNA) and concentration of dioxins (pg TEQ/g lipid) was defined as the inducibility of CYP1B1.

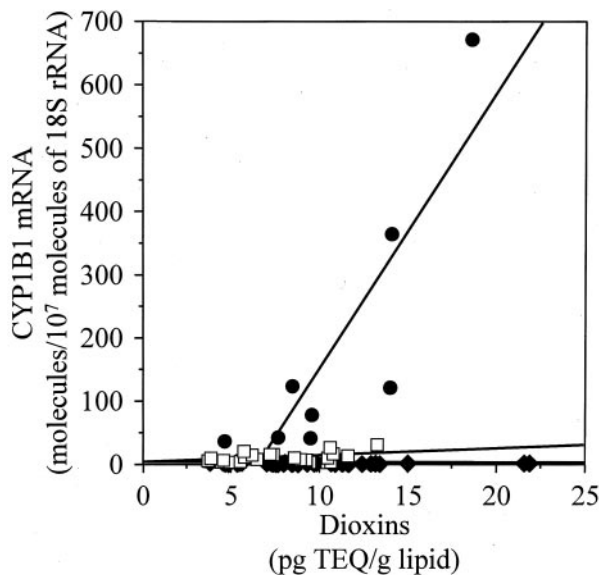
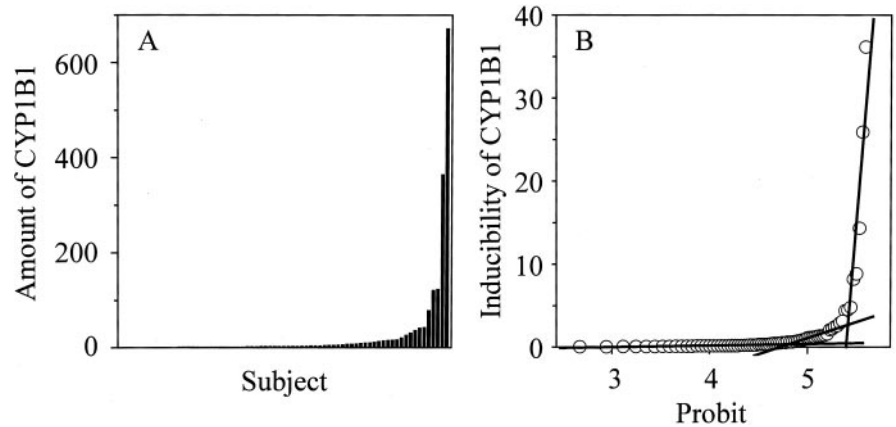


Fig. 3. The correlation between the amount of CYP1B1 mRNA and dioxin concentration in each inducibility group. A significant correlation was observed in intermediate ( $r = 0.412$ ,  $P = 0.041$ ) or high inducibility group ( $r = 0.861$ ,  $P = 0.006$ ), respectively.

in CYP1B1 mRNA has been observed *in vitro* (12). The mechanism(s) responsible for the polymorphic inducibility of CYP1B1 mRNA in human lymphocytes is still unclear at present. However, according to the previous concept (3–5), it may be possible to assume that the high inducibility subjects have higher risk to cancers caused by environmental carcinogens, such as PAHs.

The correlation between the amounts of CYP1B1 mRNA and concentration of dioxins was observed in both intermediate ( $P < 0.05$ ) and high inducibility groups ( $P < 0.01$ ; Fig. 3). The results also suggest an apparent threshold of dioxin concentration for the induction of CYP1B1 mRNA in human leukocytes. This threshold was calculated to be 6.5 pg TEQ/g lipid. This is the first to demonstrate the apparent threshold of the concentration of dioxins showing an influence in humans *in vivo*.

In conclusion, no expression of CYP1A1 mRNA and the polymorphic expression of CYP1B1 mRNA were observed in human leukocytes prepared from 72 subjects exposed to dioxins. The inducibility of CYP1B1 mRNA defined as the ratio of

CYP1B1 mRNA to the plasma concentration of dioxins showed trimodal distribution similar to those of reported inducibility of AHH activity, suggesting that CYP1B1 is the major isoform involved in AHH activity in human lymphocytes.

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