EXPRESSION OF CYTOCHROMES P450 1A1, 1A2 AND 1B1 ARE CORRELATED WITH DIOXIN LEVELS IN HUMAN LIVER

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Introduction

Dioxins modulate the expression of a number of genes including cytochrome P450 1A1 (CYP1A1), cytochrome P450 1A2 (CYP1A2), and UDP glucuronosyltransferase 1A6[1]. Detection of simple responses such as dioxin-dependent changes in the expression of CYP1A1, CYP1A2 and CYP1B1 have been linked to subsequent more complex dioxin-mediated changes such as increased levels of altered hepatic foci[2]. The possibility of the occurrence of similar responses in humans has led investigators to measure gene expression as possible biomarkers. In order to be useful, biomarkers should be detectable in easily accessible tissues. They should also be correlated with both dioxin dose and subsequent health effects. This paper reports the correlation between dioxin concentrations and the expression of several potential biomarkers in a target tissue, human liver. Although CYP1A1 and CYP1B1 expression can be induced in human peripheral blood mononuclear cells incubated *in vitro* with TCDD[3,4], it has proved more difficult to establish the dosimetric relationship between biomarkers and in vivo exposures. Previous work indicated that CYP1A2 expression in human livers was significantly correlated with the total TEQ[5]. The current paper presents two additional potential biomarkers, CYP1A1 and CYP1B1 and examines their correlation with internal dose expressed as total TEQ, only TCDD, only PCDDs, only PCDFs or only PCBs.

Materials and Methods

Human liver samples

Liver specimens from anonymous donors were obtained from two tissue banks in the early 1990's and stored at –70 °C until analysis.

Analysis of dioxin congeners

Liver tissue was analyzed by high resolution gas chromatography/mass spectrometry as previously described for 8 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), and 4 coplanar polychlorinated biphenyl congeners (PCBs)[6]. All of the reported results are adjusted for tissue lipid content.

RNA extraction

Total RNA was extracted from approximately 100 mg of frozen liver tissue using Tri reagent (Sigma Chemical Co., St. Louis, MO) as previously described[5].

Analysis of gene expression

Measurement of gene expression was accomplished by quantitative 'competitive' reverse transcriptase-polymerase chain reaction (RT-PCR), which titrates recombinant competitors against 100 ng test RNA. Vanden Heuvel[7] developed this procedure with modifications as described by Masten[3]. The assays for the detection of b-actin and CYP1B1[4], CYP1A1[8] and CYP1A2[5] have been previously described.

Analysis

The results were analyzed using SPSS version 10.0.0. TEQs were calculated using the WHO-recognized TEFs[9]. All variables except "b-actin" required log-transformation to achieve normality for Pearson's correlation analysis.

Results and Discussion

Biomarker expression and dioxin levels were measured postmortem in liver tissue obtained from 39 donors. Relatively few demographic variables were available for this population. Twenty six of the donors were male, twelve were female and the sex was not reported for one individual. This was an older population, with a median age of 56 years with a range from 28 to 83 years old. Quantitative smoking data was not available although the accompanying datasheets reported 2 current smokers, 15 past smokers, 21 nonsmokers and 1 unknown. It was not possible to assess the accuracy of the smoking data. The ethnicity of the cohort was primary white/European with 1 reported Asian and 2 unknown members.

The crude results are shown in Table 1. The mean total TEQ was 121 ng/kg whereas the median was 89 ng/kg, reflecting the skewed nature of the distribution with 6 individuals exceeding 200 ng/kg. In contrast, the total TEQ measured in the serum of individuals in the United States with "background" or "non-occupational" environmental exposure rarely exceed lipid-adjusted concentrations of 30 ng/kg[10]. Of the total TEQ, PCDFs and PCDDs contributed 40-50% while PCBs contributed less than 20%. Biomarker quantitation showed relatively low levels of dioxin-inducible genes compared to the actin "housekeeping" gene which was measured as a noninducible control. CYP1A2 had the highest level of expression with a mean of 7.3×10^7 copies per mg total RNA. In contrast, mean CYP1B1 expression was only 0.03×10^7 copies per mg total RNA. The magnitude of CYP1A1 expression exceeded that of CYP1B1 by 3-4 fold while CYP1A2 expression exceeded that of CYP1B1 by approximately 20-fold.

Table 2 shows the correlation between dioxins and the biomarkers. b-actin is not considered to be a dioxin inducible gene and accordingly there was no significant association with any of the forms of dioxin dose measures.

The highest degree of correlation was between CYP1A2 and PCDDs (r=.577, p=0.000), PCDFs (r=.603, p=0.000) and total TEQ (r=.648, p=0.000). The correlation between CYP1A1 and PCDDs/PCDFs/total TEQ also exceeded r=0.400 and was highly significant. With both CYP1A1 and CYP1A2, the correlation between the biomarker and the total TEQ was most similar to that observed with the PCDFs. A lesser but still signification correlation was seen between CYP1B1 and PCDDs/PCDFs/total TEQ (r=0.347 to 0.376). All three biomarkers showed a significant correlation of 0.321 to 0.425 with PCBs. CYP1B1 but neither CYP1A1 nor CYP1A2 showed a significant correlation with TCDD alone.

Both CYP1A1 and CYP1A2, but not CYP1B1, were found to be significantly positively correlated with age (CYP1A1 r=0.346, p=0.036; CYP1A2 r=0.370, p=0.022). However, controlling for age had little effect on the magnitude or significance of the correlation between the biomarker and dose measure as seen in Table 2.

CYP1A1 and CYP1A2 show parallel patterns of reactivity to dioxins which differ from the response characteristics of CYP1B1. In addition to lower levels of dioxin-induced RNA expression,

Table 1. Exposure biomarkers and effect biomarkers measured in 39 human livers

Dose variables	mean	median	25-75 percentile	Min, max
TCDD[1]	4.6	3.5	2.6-5.7	1.1, 20.5
PCDDs[1]	46.7	33.3	22.8-67.4	8.1, 188.8
PCDFs[1]	55.3	28.8	13.6-83.2	0.5, 257.8
PCBs[1]	19.0	8.5	4.3-17.2	0, 235.9
Total TEQ[1]	120.9	89.4	89.4-177.5	8.2, 469.8
Biomarker variables				
ß-actin[2]	24	24	14-34	2.5, 54
CYP1A1[2]	1.5	0.9	0.2-2.4	0.002, 7.0
CYP1A2[2]	7.3	5.9	0.1-1.0	0.001, 2.9
CYP1B1[2]	0.03	0.02	0.01-0.05	0.001, 2.2

[1]ng/kg (lipid adjusted)
[2]gene expression x 10^7 copies per mg total RNA

Table 2. Pearson's Correlations: Classes of dioxins and potential effect biomarkers

		TCDD[1]	PCDDs[1]	PCDFs[1]	PCBs[1]	Total TEQ[1]
actin	r	*-.110*	-.163	-.147	-.196	-.183
	Sig.	.507	.321	.372	.238	.266
CYP1A1[1,2,3]	r	*.143 (.093)*	.424[4] (.341)	.513 (.546)	.321 (.272)	.522[4] (.470)
	Sig.	*.393 (.597)*	.008 (.045)	.001 (.001)	.053 (.114)	.001 (.004)
CYP1A2[1,2,3]	r	*.250 (.207)*	.577 (.528)	.603 (.702)	.425[4] (.436)	.648 (.637)
	Sig.	*.124 (.233)*	.000 (.001)	.000 (.000)	.008 (.009)	.000 (.000)
CYP1B1[1,2]	r	.367	.347	.347	.370[4]	.376
	Sig.	.022	.030	.030	.022	.018

Italicized results are not statistically significant
[1]Data were log-transformed to achieve normality.
[2]CYP gene expression levels are expressed relative to b-actin
[3]Variable is significantly correlated with age (results controlling for age are given in parentheses)
[4]n=38
[5]n=37

CYP1B1appears to be operating by a distinct mechanism by showing an overall lower level of correlation with composite measures of dioxin doses (PCDDs/PCDFs/total TEQ) compared to CYP1A1/1A2. The distinct pattern of CYP1B1 has also been reported in Sprague-Dawley rats treated with TCDD[11]. In the rat livers, CYP1B1 showed lower levels of gene induction than either CYP1A1 or CYP1A2. The shape of the dose response curve for CYP1B1 also differed from that observed with CYP1A1/1A2 reflecting a lower degree of sensitivity to low levels of TCDD treatment[11]. While

parallel human data is not available, the lesser degree of correlation to tissue dioxin levels suggests the presence of a similar pattern of response.

These results show that presumed typical ambient dioxin exposures can result in significantly elevated levels of dioxins in human livers compared to serum, most likely because of sequestration by CYP1A2. In turn, the observed tissue levels of dioxins were found to be sufficient to significantly induce a dose dependent increase in gene expression. All three of the genes previously reported to be dioxin-inducible in either animal models or human tissues *in vitro* were found to be induced by the unremarkable ambient exposures presumed to have been encountered by the tissue donors.

In summary, ambient exposures to dioxins are sufficient to induce the expression of the potential biomarkers, CYP1A1, CYP1A2, and CYP1B1 in a target tissue. The development of a truly functional biomarker is dependent upon the ability to measure induction of these genes in a suitable and accessible surrogate tissue such as peripheral blood mononuclear or buccal cells.

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