EVIDENCE OF HEPATIC SEQUESTRATION OF DIOXINS IN HUMANS? AN EXAMINATION OF TISSUE LEVELS AND CYP1A2 EXPRESSION

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Introduction

Studies of humans and animal models show that the liver is an important organ for dioxin toxicity. Epidemiological studies of highly exposed populations have found abnormal levels of liver enzymes after acute exposures[1] and increased incidence in hepatobiliary and extrahepatic cancers among females[2]. The use of animal models makes it possible to study the pathology and mechanism of hepatotoxicity. Rodent models have shown that induction of cytochrome P4501A2 (CYP1A2) results in sequestration of dioxins in the liver[3]. Because of this, hepatic doses are higher than doses to other potential target organs. Rats treated chronically with TCDD show dose-related promotional changes within the liver including an increase in cell proliferation and preneoplastic foci[4,5]. Continuous exposure is necessary for the maintenance of these events[6].

This study addresses the question of hepatic sequestration in humans by measuring dioxin levels and CYP1A2 expression in human liver tissue. Higher than expected dioxin concentrations in the liver accompanied by a corresponding elevation in CYP1A2 expression would be consistent with hepatic sequestration. Expected concentrations of dioxins are based upon the levels measured in the serum lipids of a population having only environmental exposure. Evidence of hepatic sequestration of dioxins in humans will have important implications for risk assessment because body burdens as currently estimated though the measurement of dioxins in serum lipids may significantly underestimate the tissue levels in a target organ.

Methods and Materials

Liver tissue was analyzed by high resolution gas chromatography/mass spectrometry as previously described[7]. The analytes consisted of 8 polychlorinated dibenzo-*p*-dioxin, including TCDD, 10 polychlorinated dibenzofuran and 4 coplanar polychlorinated biphenyl congeners. Liver tissues obtained from 2 tissue banks in the early 1990's were stored at –70 C until extraction. Approximately 100 mg of frozen liver tissue was homogenized in 1 ml Tri reagent (Sigma Chemical C., St. Louis, MO) over ice. Total RNA was extracted using the method as previously described[8]. Modifications included an additional initial centrifugation to remove insoluble matter and an additional extraction with Tri reagent. Actin and CYP1A2 gene expression were measured by quantitative 'competitive' RT-PCR (RT-PCR). Recombinant competitors were titrated with 100 ng RNA as previously described.[9, 10, 11] An assay for CYP1A2 was developed using a specific primer for reverse transcription (ctgtatctcagg) and a forward and reverse primer set that spanned the location of a known intron (Forward primer: tccctgagagtagcgatgaga; Reverse primer: tgactgtgtcaaatcctgctc). The corresponding internal standard incorporates a T7 promoter site for use *in in vitro* RNA transcription and is 109 bp smaller than the 341 bp CYP1A2 gene amplicon.

The results were analyzed using SPSS version 8.0.0. TEQs were calculated using the WHO-recognized TEFs[12]. The variables, 'total TEQ' and 'TCDD' were log-transformed. The dependent variable, CYP1A2, was expressed either as copies of CYP1A2 per μg RNA or normalized by dividing CYP1A2 copies by the number of actin copies and multiplying by 10,000.

Results and Discussion

The liver tissue samples analyzed in this study were autopsy samples from two tissue banks. As seen in Table 1, Cohort 1 is substantially younger and there is very little overlap in age between the two cohorts. Cohort 1 also has significantly lower tissue levels than Cohort 2 whether measured as TCDD or by total TEQ levels. The differences between the two cohorts made it necessary to consider whether they were

Table 1. Human liver tissue: demographic attributes, dioxin tissue levels and gene expression

		Cohort 1	Cohort 2	T-test[d]	Combined
N		14	25		39
Male: Female		10:3[a]	16:9		26:12
Age (years)	Median	46	64	p=0.000	56
	25-75 %-ile	41-55	52-72		46-69
	Low, high	33: 66	28: 83		
TCDD[b]	Median	3	4	p=0.031	4
	25-75 %-ile	2-5	3-6		3-6
	Low, high	1.1: 6.5	1.7:20.5		
Total TEQ[b]	Median	46	148	p=0.000	91
	25-75 %-ile	30-59	79-205		50-177
	Low, high	8: 92	27: 470		
Actin[c]	Median	23	24	NS[e]	24
	25-75 %-ile	15-37	14-29		14-39
	Low, high	7.4: 54	2.6: 44		
CYP1A2	Median	0.08	0.87	P=0.000	0.59
	25-75 %-ile	0.03-0.18	0.59-1.4		0.11-1.0
	Low, high	0.00-0.49	0.12-2.8		0.001-2.8

[a] missing gender data for 1 individual
[b] ng/kg (lipid adjusted); log transformed data used for statistical analysis
[c] gene expression x 10^7 copies per μg total RNA
[d] t-test comparison of Cohort 1 and Cohort 2 means
[e] NS: not significant

homogeneous for a combined analysis. The decision to combine the two cohorts was based on the similar levels of actin gene expression. The lack of significant differences in this "housekeeping" gene suggested that the observed differences were not due to variation in storage or transport. Hence, subsequent analyses are based upon the combined cohort.

For the combined cohort, the mean total TEQ was 124 ng/kg with a median of 91 ng/kg lipid adjusted. Two individuals had total TEQs in excess of 400 ng/kg and an additional 3 individuals had tissue levels which exceeded 250 ng/kg. Serum levels for this cohort were not available but the liver tissue levels can be compared to other populations having environmental exposure. A

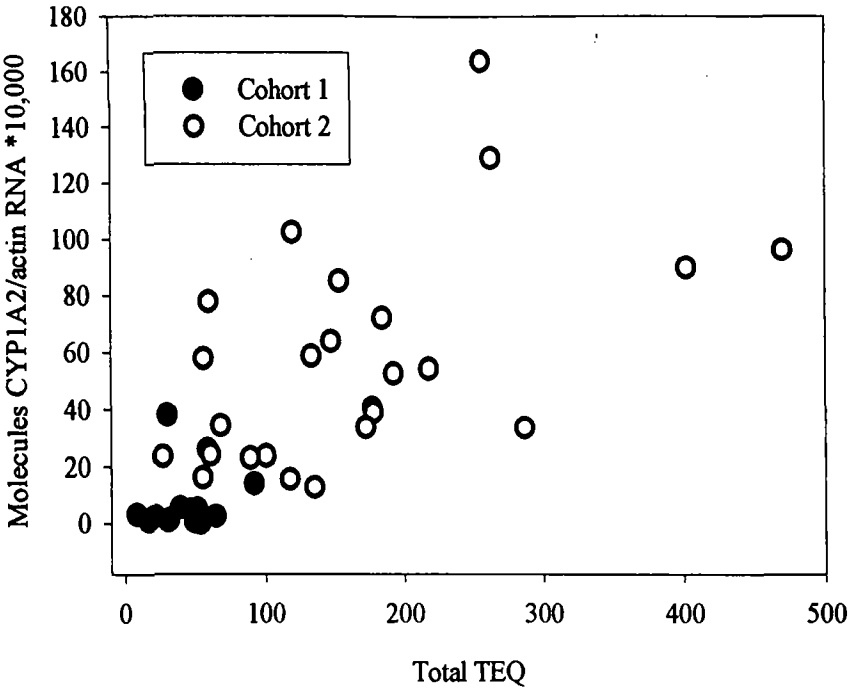

Figure 1. The relationship between total TEQ and CYP1A2 expression in 39 human livers.

Recent study of serum levels collected in 1996 shows that among a cohort of 43 individuals, median serum levels were 16 ng/kg with a range from 5 to 33 ng/kg lipid adjusted[13].
The median concentration of TCDD alone in the liver tissue was 4 ng/kg, slightly higher than the median of 1.3 ng/kg measured in the serum of individuals with environmental exposures[13].
Overall, the contribution of TCDD to the total TEQ in liver tissue ranged from 1 to 11%.
Figure 1 shows the crude relationship between dioxin levels and actin-normalized CYP1A2 expression. CYP1A2 expression is highly correlated with the total TEQ with an R^2 of 0.755 and p-value of 0.000. In contrast, the relationship between actin normalized CYP1A2 expression and TCDD has an R^2 of 0.295 and a p-value of 0.069. A potential confounder in the relationship between total TEQ levels and CYP1A2 expression is age. The crude correlation between age and actin-normalized CYP1A2 had an R^2 of 0.382 and a p-value of 0.018. However, this relationship disappeared after controlling for exposure. When the correlation between total TEQ and actin-normalized CYP1A2 was corrected for age, the strength of the relationship was slightly reduced to an R^2 of 0.679 with a p-value of 0.000.

A multiple regression analysis of CYP1A2 expression was performed and is shown in Table 2. Variables included in this model include age, gender, cohort, and actin. Unfortunately, neither smoking nor race could be included in this model. Each cohort reported only 1 current smoker. Similarly, the cohorts are predominantly white with only 4 individuals of other ethnicities. The subsequent models reinforce the above observations; total TEQ is a more important contributor to CYP1A2 expression than TCDD levels alone. The significant contributors to the model were

tissue level and cohort. However the R^2 value of the model around 0.500 suggest that other unidentified factors also contribute to CYP1A2 expression.

Table 2. Multiple regression of CYP1A2 expression in human liver

n=37	Tissue levels as TCDD (ng/kg)		Tissue levels as Total TEQ (ng/kg)	
Model R^2		0.443		0.517
	F statistic	Significance	F statistic	Significance
age	1.339	0.256	0.973	0.331
TCDD	0.863	0.360		
Total TEQ			5.727	0.023
actin	0.504	0.483	0.729	0.400
gender	0.078	0.782	0.261	0.613
cohort	18.374	0.000	5.659	0.024
gender*cohort	0.447	0.509	0.803	0.377
model	4.116	0.004	5.533	0.001
intercept	1.454	0.237	0.500	0.485

In summary, while causation cannot be determined from a cross sectional study, it is possible to assess whether the results are consistent with several possibilities. Since neither hepatic cancer status, occupation, nor serum dioxin concentrations of these individuals are known, it is possible that their serum levels were correspondingly elevated due to metabolic disturbances, mobilization of dioxins from fat depots or occupational exposure. Significant work-related exposure is relatively rare and unlikely to be present in a high proportion of the cohort. Another alternative is that the liver tissue levels are elevated relative to their serum concentrations. While we have no direct evidence of this, comparison with other environmentally exposed cohorts shows the levels to be approximately 5 to 10-fold higher than expected. In addition, the high degree of correlation between CYP1A2 expression and tissue dioxin levels is consistent with hepatic sequestration. If this is the case, human body burdens may be higher than currently estimated based solely on serum levels.

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