Population-Based Studies of Dioxin Responsiveness: Individual Variation in CYP1A1 Levels and Relationship to Dioxin Body Burden

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Introduction

As a result of the widespread and persistent occurrence of dioxin-like compounds in the environment, all individuals carry a measurable body burden of dioxins. The body of literature describing the human effects of dioxin exposure supports the conclusion that the biochemical, physiological, and clinical *response* elicited by exposure to dioxins is likely to differ among individuals, even those with similar body burdens.

On a cellular level, the biochemical response to TCDD is mediated by the AHR and the ARNT proteins, which together modulate the expression of a diverse array of genes including CYP1A1, CYP1A2, and CYP1B1 *(1)*. Since individual biological response to dioxin is mechanistically linked to this signal transduction pathway, the AHR and ARNT and TCDD-inducible genes (i.e. CYP1A1 and CYP1B1) may be useful as markers of *responsiveness* to dioxin. An integrated approach evaluating variables which contribute to interindividual variation in response to dioxin will provide insight into the health risks expected with current levels of exposure to dioxins.

Materials and Methods

Study populations. The German chemical workers in this study were employed at a chemical plant that produced organochlorine herbicides and pesticides (including 2,4,5-trichlorophenoxyacetic acid) *(2)*. Based on previous exposure assessment and epidemiological investigations, adverse health effects including chloracne, and increased mortality and cardiovascular disease have been noted in this highly exposed population *(2-5)*. Peripheral blood for lymphocyte isolation and dioxin analysis was obtained in 1992 for 113 individuals from this cohort who participated in a medical examination at the University of Mainz. Included in this group are individuals with expected low, medium, and high dioxin

exposure. The range of plasma TCDD concentration in these individuals was 1-600 ppt lipid and the total TEQ of all PCDD/PCDF/PCB congeners ranged from 15-916 ppt lipid. The population of North Carolina volunteers consists of 44 apparently healthy male and females locally recruited in the Research Triangle Park, NC area in 1996. Each volunteer was asked to donate a blood sample and complete a questionnaire to evaluate overall health, current medications or illness, smoking history, and other possible exposures to dioxin-like compounds.

Dioxin analysis. A panel of 8 polychlorinated dibenzo-*p*-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 4 polychlorinated biphenyl (PCB) congeners were quantified in frozen serum or plasma specimens by high resolution gas chromatography/mass spectrometry as described *(6)*. The total dioxin toxic equivalence (TEQ) of each sample was calculated based on WHO/USEPA toxicity equivalency factors.

In vitro culture of peripheral blood lymphocytes. Lymphocytes were isolated by Ficoll separation from freshly drawn heparinized venous blood and cryopreserved. Cryopreserved lymphocytes were cultured for three days in RPMI 1640 medium with the mitogens phytohemagglutinin (1.25 ug/ml) and pokeweed mitogen (0.15% vol/vol), and with 10 nM TCDD or vehicle control.

Assay of CYP1A1 enzyme activity. CYP1A1-dependent conversion of ethoxyresorufin to resorufin (EROD activity) was performed essentially as described *(7, 8)*.

Quantitative analysis of mRNA levels by "competitive" RT-PCR. Construction of recombinant RNA competitors and competitive RT-PCR were performed as previously described *(9)* with minor modifications *(8)*.

Results and Discussion

Experimental approach for evaluation of dioxin biomarkers and responsiveness. Our integrated approach for the evaluation of dioxin biomarkers and responsiveness is shown in Fig. 1.

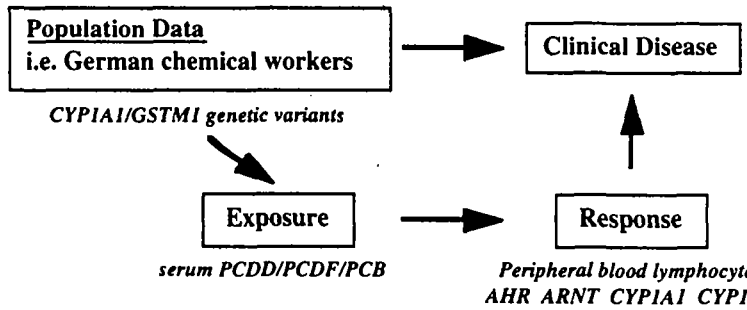

Fig. 1. Framework and experimental approach for integrated evaluation of biomarkers of susceptibility, exposure, and responsiveness to dioxins to investigate the inter-relationships between dioxin exposure and disease. The markers measured in the current studies which may influence dioxin responsiveness and hence be predictive of dioxin-associated disease are indicated in *italics*.

Our previous studies using methods developed for the quantitative analysis of AHR, ARNT, CYP1A1, and CYP1B1 in human peripheral blood lymphocytes showed that these markers are expressed in a time- and mitogen-dependent manner *(8)*. The broad objectives of our current studies are to; 1) Investigate dioxin responsiveness, measured as *in vitro* TCDD-inducibility of CYP1A1, in dioxin-exposed populations; 2) Examine factors which may contribute to individual variation in dioxin responsiveness, including prior dioxin exposure; and 3) Evaluate relationships between CYP1A1 induction and different markers of dioxin responsiveness, e.g. CYP1B1 induction.

CYP1A1 levels in German chemical plant workers. TCDD-inducible CYP1A1 mRNA and associated enzyme activity in cultured lymphocytes from 84 workers is shown in Table 1. The range of variation in CYP1A1 mRNA and enzyme activity in the total group was 17-fold and 20-fold, respectively. When the workers were categorized based on quartile of plasma dioxin TEQ levels, there was no significant differences between the groups due to the wide range of variation, which also did not differ among plasma dioxin category. There was however, a trend for increased mean and median TCDD-inducible CYP1A1 mRNA in the high exposure categories. A similar pattern was not evident for CYP1A1 enzyme activity. There was a weak but significant positive association between TCDD-induced levels of CYP1A1 mRNA and enzyme activity (data not shown). In univariate analysis, there was no apparent relationship between CYP1A1 mRNA or enzyme activity and smoking status, age, or CYP1A1 genotype.

The reason for the variation in TCDD-inducible CYP1A1 levels in the German chemical workers is not readily apparent. In similar studies with a population with low level environmental exposure (North Carolina volunteers), the combined effect of intra-individual and experimental variability accounted for less than 25% of the total variability in CYP1A1 enzyme activity. Preliminary analysis of CYP1A1 enzyme activity in the North Carolina volunteers also showed that CYP1A1 activity was positively associated with age, but not significantly associated with gender, race, dietary consumption of fried meats, or current smoking status.

Although much is known regarding the magnitude and range of dioxin exposure in humans, the lack of knowledge regarding potential human response to dioxin creates one source of uncertainty when attempting to assess risks associated with dioxin exposure. The human individual variation in response to dioxin, measured as *in vitro* CYP1A1 mRNA or enzyme induction in this study, exceeds the standard 10-fold safety factor routinely used in risk assessment practices to account for human interindividual variation. Evaluation of dioxin responsiveness may identify highly responsive individuals at increased risk for dioxin-associated disease. The success of such an approach dictates that attempts be made to account for possible contributors to variation and also that multiple responses should be examined to identify "sensitive" individuals. From the data presented here, it is as yet unclear whether CYP1A1 inducibility is related to altered susceptibility to dioxin-associated health effects (i.e. chloracne, cancer).

The dioxin exposure of the German chemical workers studied here exceeds environmental exposures in the general population by 2-3 orders of magnitude. In contrast to this highly exposed population, the variation of individual dioxin responsiveness may exceed the range of exposure in the general population. In this case, the individual responsiveness to dioxin may be a more significant determinant of risk than the magnitude of

dioxin exposure. An understanding of the factors which contribute to this variation in responsiveness then will allow an increased scientific basis for and confidence in estimated risks associated with dioxin exposure.

Table 1. CYP1A1 levels in German chemical workers by category of dioxin exposure[a]

		plasma dioxin category (pg/g lipid total TEQ)[b]			
	total	<56	56-118	119-230	231-916
Number[c]	84	21	17	21	20
CYP1A1 mRNA[d]					
mean (SD)	10.8 (7.1)	8.4 (5.5)	11.8 (8.9)	11.6 (7.6)	11.8 (6.2)
median	8.9	8.2	8.2	10.0	11.8
max/min	17.0	14.3	9.4	7.3	10.1
CYP1A1 activity[e]					
mean (SD)	2.7 (1.4)	2.5 (1.1)	3.3 (1.9)	2.5 (1.5)	2.8 (1.0)
median	2.4	2.4	2.6	2.2	2.7
max/min	19.6	5.9	8.8	8.8	5.8

[a]Values reflect levels measured in lymphocytes treated *in vitro* with 10 nM TCDD [b]Categories correspond to equal quartiles based on all samples with dioxin analysis (n=97) [c]Only 79/84 individuals with CYP1A1 values have plasma TEQ data [d]Values are the ratio of CYP1A1/β-actin mRNA ($x10^{-4}$) molecules/ug total RNA [e]Values are pmoles resorufin formed/minute/mg total cellular protein

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