Quantitation of the Extracellular Domain of the Epidermal Growth Factor Receptor in the Plasma of Dioxin-Exposed Individuals

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Abstract
This study examines the relationship among different levels of dioxin exposure, corresponding internally absorbed doses, and a cellular biomarker believed to be important in dioxin-related diseases. Blood and its plasma component were analyzed from 30 individuals. Some were German chemical industry workers with high blood dioxin levels and others were persons who had experienced medium exposures. Ten additional low-exposure subjects from the general population in several countries were also included. Internally absorbed doses for the various dioxin congeners present in whole blood were measured by high resolution gas chromatography/mass spectrometry (GC/MS) and then converted into International Toxic Equivalent (I-TEQ) units. An Enzyme Linked Immunosorbent Assay (ELISA) for Epidermal Growth Factor Receptor (EGFR) was carried out on the plasma. Compared to the general population, the chemical workers were observed to have statistically significantly higher levels of dioxin in their blood and significantly decreased amounts of EGFR in their plasma. This suggests that such biomarker assays as the ELISA may be used effectively as screening tests for identifying elevated dioxin blood levels.

Introduction
The polychlorinated dibenzo-p-dioxins (PCDDs) and related halogenated aryl hydrocarbons, such as the polychlorinated dibenzofurans (PCDFs) and the dioxin-like PCBs, are synthetic environmental contaminants that have been found to be highly toxic in some animal species, producing cancers, endocrine disruption, and reproductive, immuno-, hepato-, and neuro-toxicity. In laboratory animals and in mammalian cells in culture, these dioxins have been shown to be capable of disrupting a wide variety of biochemical processes which are likely to contribute to the aforementioned clinical effects. One of these biochemical processes which may be of

significance involves dioxin's effect on the epidermal growth factor receptor (EGFR).

EGFR is a transmembrane growth factor receptor of molecular weight 170 kD encoded by the c-erbB-1 gene and consists of an extracellular ligand-binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain. EGFR binds to extracellular growth factors (such as epidermal growth factor and transforming growth factor α) which cause receptor dimerization and autophosphorylation of the intracellular domains resulting in the stimulation of a cascade of growth signal transduction between the plasma cell membrane and the nucleus. Dioxins have been shown to result in a decrease in EGFR and EGFR-binding activity in the plasma membrane by a variety of mechanisms that may be tissue specific[1].

It is known that cells in culture that over-express EGFR in the plasma membrane release increased quantities of the extra cellular domain (ECD) into the extracellular supernatant through a process of proteolytic cleavage. Similarly, mice bearing tumors that over-express EGFR in the tumor plasma membrane can be found to have increased amounts of the EGFR ECD in their serum. We have previously used an enzyme-linked immunosorbent assay (ELISA) based on a mouse monoclonal antibody that is specific for the EGFR ECD to demonstrate increased quantities of the ECD in the serum of individuals with tumors that frequently over-express EGFR in their cell membrane. Therefore, in the current study, we have used the same ELISA to quantitate the EGFR ECD in the plasma of individuals with various levels of blood dioxin to test the hypothesis that individuals with higher dioxin levels would have lower levels of the EGFR ECD.

Methods

Thirty blood samples for analysis of EGFR ECD were selected from a series of samples previously analyzed for dioxin and dibenzofuran congeners by GC/MS so as to have three groups of ten samples each with higher blood dioxin levels, medium blood dioxin levels, and low blood dioxin levels expressed as dioxin toxic equivalents (TEQs). The high blood dioxin samples came from German chemical industry workers with known dioxin exposures and the medium and low blood dioxin samples came from the general population including some from non-industrial Southeast Asian countries. Samples had been collected by routine venipuncture techniques using dioxin-free containers and stored frozen at -20°C until analysis. Dioxin analyses were performed by GC/MS as described previously for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans[2]. TEQs were calculated for each whole blood sample using the International Dioxin Toxic Equivalence factors (I-TEQ) for each of the various congeners[3,4]. To test for statistically significant differences among the three dioxin exposure groups, the TEQ scores were log-transformed. For the low dioxin group, the blood TEQs ranged from 3.4 - 10.0. For the medium dioxin group, the blood TEQs ranged from 15.6 - 60.1. For the high dioxin group, the blood TEQs ranged from 318.0 - 672.8. Samples were analyzed for the EGFR ECD as previously described[5].

Results

Table 1 shows the relationships among occupational exposure to dioxin, its detection in workers' blood, and a commensurate biological effect in the cellular proteins present in their plasma. Compared to the general population, those exposed to high levels of dioxin had far greater levels of the chemical in their blood. The amount of the chemical was roughly proportional to their exposure level. A similar dose/response was observed in terms of dioxin's biological effect: as expected, the concentration of plasma EGFR was inversely proportional to the level of TEQ in blood. This suggests that dioxin's *in vitro* effects may also apply to exposed human populations.

Table 1.

Dioxin Exposure	Dioxin Blood Levels (Log_{10} TEQ)	EGF-r Blood Levels (fm/mL)	
High	**2.63***	**45.39[a]**	μ
	0.09	26.32	σ
	2.5-2.83	0.35-100.01	range
	10	10	N
Medium	**1.51***	**40.7***	μ
	0.19	22.9	σ
	1.19-1.78	ND-81.18	range
	10	10	N
Low	**0.77**	**73.14**	μ
	0.14	43.05	σ
	0.53-1.00	ND-132.98	range
	10	10	N

* $p < 0.05$, compared with "**Low**" exposure cohort in *t*-test

[a] $p < 0.05$, if "**High**" and "**Medium**" cohorts contrasted as single exposure cohort with the "**Low**" exposure group.

Discussion and Conclusions

More traditional industrial hygiene measurements of chemicals in the workplace are most valuable in identifying sources and amounts of potentially harmful substances. The development of sensitive and specific measurements of the "internally absorbed dose" of these chemicals can effectively integrate the total uptake of such chemicals from myriad and sometimes episodic sources. GC/MS assays can provide such internal dosimetry on body fluids or tissue in a sensitive and congener specific basis. GC/MS analysis usually requires at least 100 mL of blood whereas the technique used here requires less than 1 mL of blood. Human blood samples are particularly useful in this regard, when "steady-state" body burdens of these chemicals can be expected. Such biological sampling can be done cheaply, quickly, simply and non-invasively.

Particularly, when combined with observational epidemiology, measures of important biological effects that correlate with internal doses can make a more direct causal connection between external exposures, internal dose, and occupationally- or environmentally-related diseases. Commercially available assays such as the ELISA kit used in this study have been proven to be effective in measuring small sample volumes of body fluids such as human urine and blood for changes in such biologically crucial proteins as EGFR. Combined with clinical follow-up, these newly emerging bioassays may provide effective early warning systems or screening tools for occupationally and environmentally related exposure and disease.

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