RISK IV

Estimating variability in response to dioxin after exposure in Seveso, Italy

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Abstract

Twenty years ago near Seveso, Italy, an explosion at a trichlorophenol plant released approximately 1.3 kg of dioxin into the surrounding countryside. Compared to industrial scenarios, the affected population was diverse because it included a substantial proportion of children and women. Subsequent clinical tests have indicated no abnormalities but questions remain regarding whether some individuals may be at a high risk of cancer, and also immunological or endocrine abnormalities.

We are presently examining inter-individual variability in the interaction of dioxin with the Ah receptor (AhR) and the genes that it regulates. While binding to the AhR is essential for subsequent cellular events, it has not been examined in exposed human populations. Estimation of the variability due to inter-individual differences in response requires that variability from other sources be measured or controlled. Variability due to differences in exposure will be accounted for by measuring dioxin in the blood, which has a half-life greater than 7 years. Intra-individual variability will be estimated with serial samples from a group of North Carolina volunteers. Repeated analysis of a group of samples will be used to measure procedural variability due to fluctuating culture and assay conditions. Finally, the variability in response measured in people from Seveso will be compared to the responses of populations with environmental and industrial exposures.

The results will be used to determine the degree to which individual responses to dioxins differ and whether these differences are related to age, sex, or selected characteristics such as percent body fat.

Introduction

Following the accident in Seveso, highly exposed populations from the surrounding area were identified and their health status continues to be monitored. A number of individuals developed chloracne and early studies detected an increase in liver enzyme induction (Ideo et al., 1985). Later clinical evaluations revealed no abnormalities (Assennato et al., 1989; Mocarelli et al., 1986). However, after ten years, excess hepatobiliary, thyroid and soft tissue sarcomas began to be detected (Bertazzi et al., 1989).

Studies of the mechanism of dioxin's activities indicate that virtually all changes mediated by dioxin are preceded by binding to the AhR which regulates the expression of a number of genes including cytochrome P450 1A1 (P450 1A1), P450 1A2, P450 1B1 and glutathione s transferase M (GSTM). When animals are treated with dioxin, they develop abnormalities in several organs including the thyroid, lung, and liver (Lucier et al., 1993). Treatment also alters immune and endocrine function (Huff et al., 1994).

Cytochrome P450's have previously been reported to be induced by exposure to environmental contaminants. Women exposed to polychlorinated biphenyls and furans (Clark et al., 1992), or known to consume smoked meats, cheese, fish or smoke cigarettes (Whyatt et al., 1995) have been found to have highly induced P450 1A1 activities in either their own or their placental tissues. P450 1A2 has been found to be induced by cigarette smoking (Butler et al., 1992) and consumption of pan-fried meats (Sinha et al., 1994). Induction of cytochrome P450 1B1 has not been examined in humans but rodent models indicate a role in the metabolism of estrogens (Spink et al., 1995).

In many respects, people from Seveso are an ideal population for examining variability in dioxin responsiveness. They were exposed only to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), and the source and timing of the exposure are known. Internal dose can be measured in either adipose tissue or the blood, reducing the opportunity for misclassification of exposure status (Bertazzi et al., 1989). In addition, the population includes all ages and similar proportions of men and women.

Variability in the early events following exposure to dioxin implies that differences in susceptibility to adverse health effects may exist. This is further supported by observed differences in the health impact of dioxins. Intact female rats show a higher degree of oxidative damage (Tritscher et al., 1996) and increased incidence of liver tumor than males or ovariectomized female rats (Kociba et al., 1978; Huff et al., 1994). Observations of primates suggest that dioxin may play a role in the development of endometriosis (Rier et al., 1993). Children from Seveso also appeared to be more prone to developing chloracne than similarly exposed adults (Mocarelli et al, 1991).

Assessment of response variability to critical and universal events following exposure will be a first step in identifying susceptible individuals and populations.

Approach

1. Develop biomarkers capable of detecting dioxin-induced alterations.

Dioxin responsiveness is assessed in lymphocytes that are mitogen stimulated and treated with 10 nM of TCDD. Preliminary work showed that mitogen stimulation is necessary for the expression of the AhR in human lymphocytes (Clark et al., in press). The low level of P450 1A1 activity in resting lymphocytes is also augmented by mitogen stimulation.

Because dioxin's toxicity is mediated through the AhR, we have developed assays to detect AhR, and several enzymes which it regulates. They are shown in the analysis scheme presented in Figure 1 and include P450 1A1, P450 1A2, P450 1B1, glutathione S transferase M and UDP glucuronyltransferase. Whenever possible, both mRNA and protein are measured. While very low numbers of specific mRNA molecules can be detected, evidence of the production of protein is a better indicator of biologically significant gene activity. Gene-specific probes and PCR are used to measure the steady state levels of mRNA. An AhR-directed antibody will be used to measure AhR protein levels by Western blotting. The catalytic activity of cytochrome

P450 1A1 will be measured by incubating lymphocytes with the substrate ethoxyresorufin.

The timing of the lymphocyte harvest from culture was optimized by assaying the cells daily for a week. The protein-related measures, AhR detected by Western blot and measurement of P450 1A1 catalytic activity, peaked at day 3, while the mRNA for AhR peaked on day 2.

2. Lymphocytes as a surrogate for target tissue

Lymphocytes act as a surrogate tissue for events occurring in target tissues such as the liver. To assess whether the lymphocyte responses are comparable to a target tissue, mRNA levels for P450 1A1, P450 1B1 and AhR will be measured in hepatic tissue obtained postmortem from a cohort of 48 accident and cancer patients. In addition, mRNA for P450 1A2 and glucuronyltransferase, which are not expressed in lymphocytes, will be measured in the liver tissue.

3. Estimate response variability due to interindividual differences

Figure 2 shows the general strategy for distinguishing inter-individual from other sources of variability. Variability will be assessed for each endpoint using resting, mitogen-induced,and mitogen-induced dioxin-treated lymphocytes.

The contribution of dioxin exposure to the total variability will be assessed by measuring the variability in blood dioxin levels. An estimate of intra-individual variability will be obtained from North Carolina volunteers by analysis of 3 serial samples taken at weekly intervals. Procedural variability will be measured by repeatedly analyzing pooled lymphocytes from at least three individuals throughout the course of the analysis.

The above strategy will be used to estimate the inter-individual variability for three cohorts: 1) 91 Seveso residents with their very high acute exposures 2) 20 approximately North Carolina volunteers which have low levels of environmental exposure 3) 109 Boehringer industrial workers, which have high level, long duration (up to 30 years) exposure to dioxins.

Potential effect modifiers such as GSTM1 status, smoking status, age, race and sex will be evaluated for their impact on response variability.

Summary

Continuing collaborations will compare the biomarkers described above in populations whose illness suggests heightened susceptibility to dioxins. In addition, the dioxin-responsive biomarkers will also be applied to animal models to study variability in susceptibility to endocrine dysfunction, immunological effects and the development of cancer.

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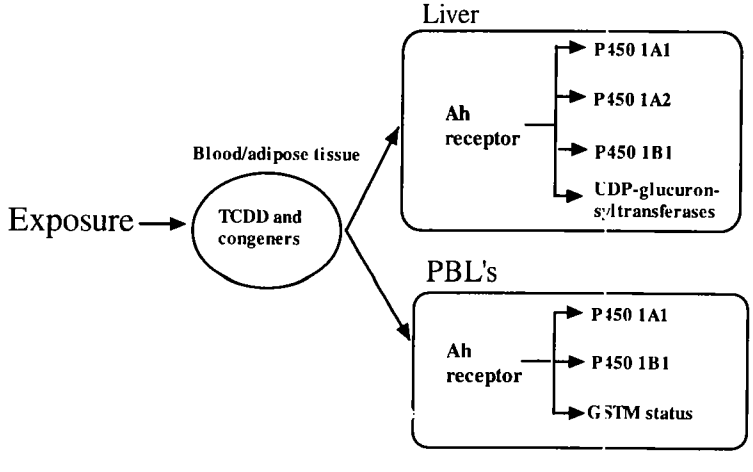

Figure 1. Summary of the dioxin-relevant endpoints that will be measured in lymphocytes and liver.

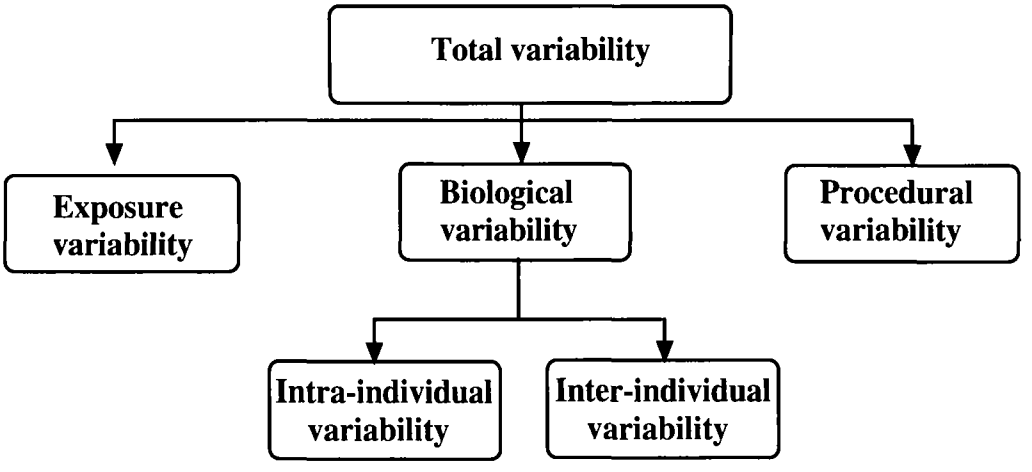

Figure 2. Strategy for estimating the variability due to inter-individual differences in response.