DIOXIN RECEPTORS AND TOXICITY

Lucier, G.W.[A], Tritscher, A.M.[A], Vanden Heuvel, J.P.[B], Sewall, C.H.[A], Portier, C.J.[A], Clark, G.C.[A]

[A]National Institute of Environmental Health Sciences, Research Triangle Park, NC, U.S.A.
[B]Purdue University, West Lafayette, IN, U.S.A.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) or dioxin, as it is frequently called is produced inadvertently during the synthesis and incineration of other chemicals in the presence of chlorine. It is extraordinarily toxic with a range of LD_{50} values from 1 μg/kg in guinea pigs to 5000 μg/kg in hamsters. TCDD produces a number of toxic effects in experimental animals including teratogenicity, reproductive toxicity and immunotoxicity.[1] TCDD is also a potent carcinogen in animals[2] and most risk assessments for TCDD are based on the carcinogenic effects in rat liver.[3] The mechanism responsible for TCDD's carcinogenic effects are not fully known although TCDD is negative in short-term tests for genetic toxicity and it does not form DNA adducts. Moreover, TCDD is a potent tumor promoter and a weak initiator in two-stage models for skin[4] and liver[5,6] cancer. Since it is generally accepted that most, if not all, of TCDD's effects are dependent on an initial interaction with the Ah receptor, TCDD's toxic effects are considered to be receptor-mediated.

The generally-accepted mechanism for Ah-receptor mediated events involves a series of steps. First, TCDD or its structural analogs bind the Ah receptor with high affinity and selectivity. This is followed by dissociation of other protein(s) and association with others (i.e. arnt protein) which permits binding to responsive elements on specific genes and subsequent changes in gene expression.[7]

There is considerable controversy on the dose-response relationships for TCDD's effects and the choice of approach for estimating human risks from animal data. Our research has focused on the characterization of dose-response relationships for Ah receptor dependent biochemical effects (induction of CYP1A1 and CYP1A2, and loss of plasma membrane EGF receptor) following administration of TCDD to female rats (100 pg/kg/day to 125 ng/kg/day) for 30 weeks within the framework of a two-stage model for hepatocarcinogenesis.[6,8] Dose-response relationships for biochemical effects were compared to those for coordinated biological responses such as rates of cell replication and the size of putative preneoplastic lesions (foci of cellular alteration). We have also evaluated the relevance of animal data for predicting human responses to dioxin and its structural analogs,

and we have examined some of the possible mechanisms responsible for interindividual variation in human responses to TCDD.

Dose-Response Relationships for TCDD's Effects

Induction of CYP1A1 and CYP1A2 in liver preparations was quantified by radioimmunoassay. Dose-response relationships were determined in relation to both administered dose and the concentration of TCDD in livers.[8] Data revealed that the ED_{50} for both CYP1A1 and CYP1A2 induction was approximately 10 ng/kg/day or 2 ppb dioxin in liver. Increased CYP1A1 concentrations were statistically significant at a dose of 100 pg/kg/day whereas a dose of 1000 pg/kg/day was necessary to produce a statistically significant increase in CYP1A2 concentrations. This difference most likely reflects the higher amount of CYP1A2 in control livers, not a true difference in sensitivity. It is also important to note that limit of detection of any response should not be confused with a threshold. Our dose-response data for CYP1A1 and CYP1A2 induction were analyzed by the Hill equation which can detect linearity or non-linearity in response over a wide dose range. These analyses revealed that the best fit to the data was a model which predicts a proportional relationship between liver concentration and induction which means that these data are most consistent with a linear response. Although there is no clear mechanistic link between CYP1A1 and CYP1A2 induction and toxicity, many of the chemicals that induce these P-450 isozymes are carcinogens. Dose response relationships for TCDD-induced loss of hepatic plasma membrane EGF receptor were also evaluated in the same two-stage model for hepatocarcinogenesis. Mathematical analysis of this data indicated that the best fit was a linear relationship between target tissue dose and response.[9] Moreover, a mechanistic model for TCDD's effects on the EGF receptor predicted a linear relationship between target tissue dose and response.[10] Internalization of the EGF receptor is thought to represent an early step in stimulation of hepatocyte mitogenesis. TCDD's effects on the EGF receptor and cytochrome P-450 isozymes have been shown to be Ah receptor dependent.

Dose response relationships for coordinated biological responses such as TCDD-mediated increases in cell proliferation were different than those for effects on cytochrome P-450 isozymes or the EGF receptor. For example, effects on cell proliferation were highly variable. Approximately 50% of the rats in the high dose group (125 ng/kg/day) had significantly elevated cell proliferation rates whereas the other half had rates similar to controls. Also, there was no evidence of enhanced cell proliferation rates in livers of rats receiving low doses of TCDD. Evaluation of foci of cellular alteration (preneoplastic lesions) in the same livers indicated that this response, like cell proliferation, was highly variable and not detectable in the low dose group. Taken together, our dose response data demonstrate that the shape of the dose-response curve cannot be predicted simply on the basis that a response is Ah receptor-mediated. This conclusion is consistent with the knowledge that some steroid and peptide hormone receptors produce qualitatively and/or quantitatively differences in response in different cells, and that these differences are related to cell specific factors such as receptor isoforms, location of responsive elements on DNA and interactions with other proteins that modify or guide responses.[11]

Relevance of Animal Models for Estimating Human Risks

There is a growing agreement that humans respond similarly to TCDD and its analogs as experimental animals. Human cells appear to contain a fully functional Ah receptor. Also, TCDD and its structural analogs produce many of the same changes in Ah receptor-dependent gene expression as observed in experimental animals. For example, placentas of Taiwanese women exposed to rice oil contaminated with polychlorinated dibenzofurans exhibited markedly elevated levels of CYP1A1 and growth factor pathways were also altered in these tissues.[12] Comparative dosimetry analyses demonstrated that humans are at least as sensitive as rats to these effects. Likewise, several studies have shown that human and animal cells in culture respond to TCDD in a similar way. The sites of human cancer, reported in recent epidemiological studies, are in general agreement with animal cancer sites and these studies indicate that TCDD is a multisite carcinogen at doses well below the maximum tolerated dose. There is now a legitimate debate over the shape of the dose response curve for toxic effects of TCDD especially in the low dose region. Our lack of knowledge concerning the entire sequence of events responsible for the myriad of dioxin's toxic effects hinders our ability to clarify dose response relationships for toxic effects. We also need to understand the mechanism responsible for TCDD's interactions with other endocrine systems such as estrogens and pituitary hormones. This information would permit more careful evaluations of both non-cancer and cancer effects of TCDD.

Interindividual Variation

It is becoming apparent that there is considerable interindividual variation in TCDD's effects on humans. For example, some individuals, accidentally-exposed to a given level of dioxin develop chloracne whereas other individuals exposed to the same amount of dioxin do not. There is also considerable interindividual variation in the CYP1A1 induction by TCDD and its structural analogs in humans. In collaboration with the National Cancer Institute, the National Institute of Occupational Safety and Health, German scientists and Italian scientists we are attempting to determine mechanisms responsible for sensitivity or resistance. Our preliminary efforts have quantified the amount of Ah receptor by photoaffinity labeling in human lymphocytes and the data suggest that amounts of Ah receptor, may in part be responsible for differences in human responses to dioxin.[13] We are also investigating mutations in dioxin responsive genes that confer high or low responsiveness to dioxins.

Summary

In summary there is growing agreement that the broad spectrum of toxic and biochemical effects produced by dioxin require an initial interaction with the Ah receptor which after several steps leads to activation or repression of critical target genes. It is also becoming clear that dose-response relationships for dioxin's effects cannot be predicted solely on the basis that a response is receptor-mediated. There is increasing evidence that the Ah

receptor functions in a similar way in humans and experimental animals and that animal and human data are appropriate for risk assessment.

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