Does The Exposure To High Levels Of 2,3,7,8-Tetrachloro-Dibenzo-*p*-Dioxin (TCDD) Cause Perinatal Imprinting Of The Inducibility Of Cytochrome P4501a2 Activity In The Human?

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Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and dioxins such as TCDD are polyhalogenated aromatic hydrocarbons (PHAHs) that are ubiquitous environmental chemicals. These PHAHs are known to alter many biological functions and cause adverse effects in the human and other mammalian species. One of the most studied biologic response is the capacity of TCDD, PCBs and PCDFs to alter the expression of Cytochrome P450 family 1 enzymes in animals and humans.

The Cytochrome P450 family 1 enzymes have been studied primarily because the induction of the enzyme family by the PHAHs appear to occur at body burdens lower than most if not all of the other biologic systems that are altered by the PHAHs, and the degree that the P450 family 1 enzymes are induced seem to correlate with the potential toxicity of the PHAHs to animals (1) and to humans (2). Also, the susceptibility of the species to the toxicity of the PHAHs in general and specific congeners roughly correlates with the degree these PHAHs can induce Cytochrome P450 family 1 (1,2).

Cytochrome P450 family 1 has three enzymes, Cytochrome P450 1a1, 1a2, and 1b1. Each P450 family 1 member is expressed in tissues with developmentally specific pattern. P450 family 1 enzymes are primarily regulated by the Ah (aryl hydrocarbon) receptor with Cytochrome P4501a2 being primarily expressed in the liver. We have previously reported that Cytochrome P4501a2 activity is induced by dioxin, PCBs, and PCDFs in the human and their induction correlates with their known toxic effects in the human (2).

Children in many ways do not respond to the PHAHs as adults. Children are more sensitive to the toxic effects of many of the PHAHs including PCB/PCDF mixtures, PCBs by themselves, and

possibly even dioxin. There appears to be a sensitive window of increased susceptibility in the developing human and, contrary to the adult, the child's Cytochrome P4501a2 activity does not appear to be induced by high body burdens of any of the PHAHs. The study reported here addresses the question of whether high body burdens of TCDD during development may imprint the Ah receptor-Cytochrome P4501a2 system complex resulting in the alteration of the expression and /or inducibility of the children's Cytochrome P450 1a2 activity when they become adults.

The cohort utilized in this study was the Seveso cohort of TCDD exposed subjects. These subjects were exposed to very high levels of TCDD in 1976 following an industrial accident that released the TCDD into the residential environment surrounding the factory.

Cytochrome P4501a2 activity in the human subjects can be sensitively, safely, and non-invasively monitored by the carbon thirteen labeled [3 methyl] caffeine breath test (CBT). The CBT monitors only Cytochrome P4501a2 dependent 3 N-demethylation of caffeine (3) and was employed in this study. Carbon thirteen is a stable naturally occurring isotope of carbon which is not radioactive and makes up about one per cent of all the carbon normally found in the human..

Materials and Methods

Recruitment of Subjects
Subjects were recruited from the Seveso cohort during 1992 to 1995. There were 33 females and 33 males included consecutively from exposed people coming to Desio Hospital for periodical monitoring. The subjects ranged from a few months of age to 54 years of age when the accident occurred in 1976. Inclusion criteria for the study were that all the subjects had to be healthy, did not smoke tobacco for at least two years prior to the study day ,had a TCDD measure in serum drawn in 1976 after the accident and another serum sample in 1992-1995 suitable for TCDD measurement. The study was approved by the IRB and all subjects signed the consent form prior to the study.

Caffeine Breath Test
The CBT was conducted in 1992 through 1995. The subjects came to the clinic between 8 and 10 am after an overnight fast. After sitting for 5 minutes, the subject blew into a 20 cc syringe until end tidal breath was collected. The subjects than drank 20 mL of Coke Light containing 3 mg/kg of labeled caffeine up to a maximal dose of 200 mg. The subject then blew into the syringe every 30 minutes for two hours. The captured breath was transferred to a vacutainer for storage and transported to the laboratory. The breath ratio of carbon twelve to carbon thirteen was determined by GC-Mass spectroscopy. The excess moles of carbon thirteen labeled carbon dioxide exhaled are calculated using the BMR of each subject and the increase in labeled carbon dioxide exhaled over two hours is determined. The CBT results are reported as the per cent of administered carbon thirteen exhaled over two hours (3).

Serum TCDD levels
The TCDD serum levels were performed as previously described and reported as TCDD in ppt on lipid base (4). Blood samples was taken by venipuncture in 1976 and in 1992.

Statistics
All tests comparing the CBT from one age of exposure group to another age of exposure group was conducted using the Wilcoxon rank test. The subjects were divided into 3 groups according to the subject's age at time of the accident: the Early Childhood Exposure Group consisted of subjects who were between a few months of age to 5 years of age at the time of exposure (13 subjects, 7 females and 6 males); another group, the Late Childhood and Adolescence Exposure Group who were between 6 and 16 years of age (17 subjects, 8 females and 9 males); and the third group of subjects who were adults at the time of the accident, the Adult Only Exposure Group (36 subjects, 18 females and 18 males), who were between 16 and 54 years of age when the accident occurred.

Results and Discussion
The serum TCDD levels for the females ranged between 30 and 56,000 ppt in 1976, and 12 and 800 ppt in 1992;those for the males were between 50 to 11,000 ppt in 1976 and 1 to 700 ppt in 1992. The serum levels in 1976 were higher than those observed in 1992. However, there was no difference between the different exposure groups in TCDD serum levels observed in the blood collection in 1976. Similarly there was no difference in TCDD serum levels observed in the blood collected in 1992.
The CBT, expressed as per cent of carbon thirteen label administered which is exhaled over two hours after the caffeine was taken, demonstrated some gender and age specific differences. The CBT median (and range) for females was as follows: Early Childhood Exposure Group 3.8% (1.3- 4.8%) ; Late Childhood and Adolescent Exposure Group 6.0% (3.2 – 10%); Adult Only Exposure Group 6.5 % (3.0- 15%) . The results for the males were as follows: Early Childhood Exposure Group 4.6 % (2.5 – 5.2%), Late Childhood and Adolescent Exposure Group 4.2% (2.7-6.4%), and Adult Only Group 5 % (3.0 % - 9.0 %).

The only significant differences was that the adult females from the Early Childhood Exposure Group had a decreased cytochrome P4501a2 activity in 1992-1995 ($p < 0.01$) as compared to the adult females from the Late Childhood Exposure Group and the Adult Only Exposure Group. This was observed even though the TCDD serum levels of all the groups were not different in 1976 or in 1992 when compared to each other during the same time period. If there was a trend in the TCDD serum levels, the Early Childhood Exposure Group had higher serum values of TCDD.

The observation that the adult exposed to high levels of TCDD during early childhood as compared to the adult exposed to high levels of TCDD as an older child or adult implies that this difference in inducibility may be permanent and perinatally imprinted. It is possible that there is a window of susceptibility for perinatally imprinting P4501a2 inducibility and that the window of increased susceptibility may occur beginning as early as in utero but appears to begins for sure at least during early childhood and continues during this period. We have previously reported imprinting of the Ah receptor-P4501a2 complex in the human after pregnancy and or lactation (5). The molecular reason or location of either imprinting (receptor, translocator, DNA, mRNA , etc) needs to be determined. It is of interest that imprinting appears to be during the perinatal/developmental period.

P4501a2 activity is essentially not present in humans in the immediate postnatal period and then P4501a2 is endogenously induced over the next years to levels that are twice that of the adult. The P4501a2 activity returns to the adult levels during puberty (6). It is during the process of going from no real expression of P4501a2 to levels higher then those in adults when this apparent perinatal imprinting occurs and it is precisely this change in endogenous P4501a2 levels that may make the human susceptible to perinatal imprinting at this time.

This observation of apparent susceptible window of exposure again demonstrates how the developing human responds to these chemicals in an unique manner. The molecular mechanism by which these changes occur and the relative importance of the expression of P450s and their imprinting to the overall susceptibility of the human to the PHAHs and normal development remains to be identified.

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