CYTOCHROME P4501A2 ACTIVITY IN HUMANS EXPOSED TO PCBs AND DIOXINS

Lambert G.H.[A], Schoeller D.A.[B], Hsu C.C[C], Taylor P.[B], Humphrey H.E.B.[D], Garcia F.[A], Dai L.[E], Budd M.[D], Schecter A.[F], Lietz H.[A]

[A]Loyola University School of Medicine, Department of Pediatrics, Maywood, IL 60153 USA
[B]University of Chicago, Clinical Nutrition Unit, Chicago, IL 60637 USA
[C]National Cheng Kung University, Tainan, Taiwan, ROC
[D]Michigan Department of Health, Lansing, Mi 48909 USA
[E]Hanoi Medical School, 10-80 Committee, Hanoi, Vietnam
[F]State University of New York, Department of Preventive Medicine, Binghamton, NY 13903 USA

Dioxins, polychlorinated biphenyls, polybrominated biphenyls and mirex are halogenated aromatic compounds (HAs) that are found throughout the world and in all human populations. These HAs cause similar toxic effects in mammals. However the severity of toxic effects caused by these HAs is dependent upon both the body burden and specific HAs congeners administered to the animal[1]. The toxic potential of these HAs can vary from one of the most toxic substrates known to science; 2,3,7,8 tetrachlorodibenzo-p-dioxin; to relatively non-toxic substrates such as some of the polyhalogenated biphenyls.

In animals, the induction of cytochrome P450 family 1 enzymes is a useful biomarker of HAs toxicity for several reasons. P450 1 activity is the first or one of the first biological parameters that is initially altered by the HAs at the lowest body burdens. Also the degree of cytochrome P450 1 enzyme(s) induction by these HAs directly correlates to and is predictive of the capacity of these chemicals to be toxic[1]. This correlation appears to be due to P450 1 enzyme induction being directly or indirectly involved in the HAs' mechanism(s) of toxicity. The capacity of the HAs to induce P450 1 activity in the human and whether or not the degree of induction correlates with (and predicts) the toxic capacity of the HAs in the human has never been studied.

This abstract will present an update of our work to test the following hypotheses in the human:

1] HAs can induce P450 family 1 enzyme, P450 1A2, in the human.

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2] the degree of induction correlates with the toxic capacity of the HAs.

The P450 1A2 activities of two groups of subjects and one individual uniquely exposed to HAs were investigated and compared to previously reported data from subjects with other HA exposure and their related toxicities. One group consisted of 7 adults who consumed large amounts of Lake Michigan salmon and trout for many years as compared to 7 nonfisheaters. The next group consisted of 26 adults who lived for years in the area of active spraying of dioxin containing herbicides in southern Vietnam and subjects from northern Vietnam never exposed to the chemical spraying. The single subject with unique exposure was an adult who synthesized 2,3,7,8 tetrabromo- and tetrachlorodibenzo-p-dioxins 30 years prior to our study and had recent tetrabromodioxin serum levels of 600 ppt and tetrachlorodioxin levels of 18ppt[2]. The data from these current studies were compared to the P450 1A2 activity of PBB exposed subjects from Michigan[3], or PCB/dibenzofuran exposed Yucheng subjects in Taiwan[4], or mirex[4] exposed subjects in Ohio, or control Chinese or Caucasian subjects with no unique exposure history. All the above subjects were nonsmokers. In addition; Chinese, Caucasian, or Vietnamese smokers who had no history of an unusual exposure to HAs were used as positive control groups for P450 1 induction.

The P450 1A2 activity was determined using the [^{13}C 3-methyl) caffeine breath test (CBT) as previously described[3]. The CBT monitors 3 N-demethylation which is a specific P450 1A2 dependent reaction[5]. The CBT is expressed as percent carbon label exhaled per hour over two hours. The statistical differences between the groups were calculated using the Mann-Whitney U test.

GROUP	n	serum levels median (range)	CBT [%dose exhaled/hr] median (range)
EXPOSED			
Vietnam,south	26	NC	2.7* (0.6-5.5)
Mirex[4]	9	<0.2ppb(<.2-1.1)	4.4* (2-8.9)
PCB	7	24ppb* @(5-135)	4.6* (3.1-9.7)
2,3,7,8 bromo & chlorodioxin[2]	1	600ppt# 18ppt#	4.5
PBB[3]	51	12ppb@(<1-800)	5.2* (2-11)
PCB/Dibenzofuran[4] (Yucheng subjects[6])	50	NC	16* (8.2-23.1)
CONTROL			
Chinese[4]	39	NC	3.5 (1.2-6.2)
Caucasian			
Ohio,Ill.[3]	45	NC	3.3 (0.5-9)
Mich.	7	5.9ppb@(4-8)	2.7 (0.3-5.6)
Vietnam,north	8	NC	4.1 (1.6-7.3)
Total control subj.	99		3.4 (0.3-7.3)

* p<0.05 as compared to the control subjects, @ Hexabromobiphenyl or total Arochlor 1260 serum levels, # lipid content, NC means blood not collected for congener quantitation

Results and Discussion

The PCB exposed fisheaters from Michigan had elevated PCB serum levels and CBT as compared to their control Michigan subjects, the Caucasian control subjects, and all the control subjects. The CBT was lower in the exposed Vietnam subjects as compared to the total control subjects but was not statistically different from the subjects not exposed to chemical spraying who lived in the northern part of Vietnam. Control subject groups were not different from each other. The CBT data from Chinese, Caucasian, and Vietnamese subjects who smoked were similar to one another (data not presented in the table). The median CBT value for all smokers was 5.7 (range 1.1 - 10) % dose exhaled per hour (N=80).

The CBT data of the PCB, PBB, and PCB/dibenzofuran exposed cohorts clearly indicate that these HAs can induce P450 1 function in the human adult. The southern Vietnam group exposed to spraying was not induced as compared to the control groups. This may be due to the dioxins in these subjects decreasing over time to a body burden below that which is necessary to induce P450 1 activity. Levels of dioxin currently found in south of Vietnam subjects is similar to levels found in the industrial countries. However the finding of no lower level of P450 1 activity in the north of Vietnam residents is noteworthy since they have very low dioxins levels[7]. Possibly a higher level of dioxin than seen in the US general population or the Vietnamese in the south is necessary before induction of P450 1 occurs in most humans.

The individual with high serum dioxin levels has CBT values that are in the range of the control subjects or those exposed to weak inducers of P4501A2 activity. This CBT value is far below all the Yucheng subjects exposed to the highly toxic mixture of polychlorinated biphenyls and dibenzofurans in Taiwan[6] ,and this may be due to several reasons. 1] The body burden of the HAs are higher in the Yucheng subjects than those found in the dioxin exposed individual. 2] There may be additive, synergistic or negative interactions between the various HAs and other P450 1 inducers in the subjects from the Yucheng population or the dioxin exposed individual. 3] Dioxins may not be as potent P450 1 inducers in the human in vivo as they are in the animal models. Clearly many more subjects with high dioxin levels need to be studied to determine the capacity of dioxins to induce P450 1 function in the human.

The control groups had similar CBT values indicating that the constitutively expressed P450 1 activity is similar. The Caucasian, Chinese, and Vietnamese smokers who smoked at least 10 cigarettes per day had similar P450 1 function as determined by the CBT. This indicates that the three genetically different groups had similar P450 1 inducibility, since cigarette smoke is a known P450 1 inducer. Therefore the differences in the CBT or toxicity from exposure to the HAs would not appear to be related to the genetically controlled inducibility of P450 1 in the different racial groups.

At present there is a correlation between toxicity and P4501A2 induction in the human. The most toxic exposure to the HAs was the Yucheng exposure[6] and these adults had the highest CBT values. The dioxin exposure and the PCB and PBB exposures had mild to moderate toxic effects in the human and the CBT values were just mildly to

moderately elevated. The mirex exposure may have resulted in the least amount of effects and the mirex exposed group appears to have CBT values just slightly higher than the control subjects.

The major limitation of the Vietnam and Taiwan studies is the lack of quantitation of the HAs congener specific serum levels in the subjects. Current studies are in progress to repeat the initial studies in the Vietnam subjects from the provinces with the highest levels of chemical spraying and the Yucheng population. Serum dioxin, PCB, and dibenzofuran serum levels from the Vietnam and Taiwan subjects will be analyzed.

Clearly more CBT studies need to be conducted with congener specific analysis of the HAs body burdens of the subjects and to study not only the correlation between congener specific body burdens of the HAs and P450 1 induction; but also the relationship between the HAs body burden, the induction of P450 1 activity, and the disease states found in the subjects.

In summary, the HAs are in vivo inducers of P4501A2 in the human and their degree of induction in a cohort appears to correlate with the HAs'toxic effects. The CBT may be a valuable biomarker of HAs exposure, effect, and toxicity in the human.

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