GLUTHATION-S-TRANSFERASE POLYMORPHISM AND ORGANOCHLORINE LEVELS IN A PCB CONTAMINATED AREA

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Introduction

Polychlorinated biphenyls were produced in the Michalovce district of eastern Slovakia from 1959 to 1984. More than 20,000 tons of Delors, Hydelors and other mixtures similar to Arochlors produced in the United States were produced. During the decades of the production, considerable amounts of these compounds leaked or were released into the environment and caused contamination of soil, sediments, biota, and the food web in the district [1]. Recent studies also documented elevated PCB levels in humans in this district in comparison to district with no PCB production [2-3]. PCBs were found to be toxic in animal studies and have suspected carcinogenic, hormone-disrupting and developmental effects in humans [4-5].

This study was conducted to characterize the distribution and association of biotranformation enzyme glutathion S-transferase (GST) with organochlorines in a sample of the general population from the district in the eastern Slovakia with one of the highest PCB levels documented in humans and the environment in Europe.

Methods and Materials.

Subjects, 200 males and females from the Michalovce district, were selected by systematic random sampling from the database of patients of nine primary care physicians. This database covers a specific geographic area assigned to the physician and provide an almost complete population list. In 1998, 10 ml of blood was obtained for the organochlorine analyses and 3ml of the whole blood was collected from each participant kept frozen until the time of analysis. Fifteen mono-ortho and di-ortho PCB congeners (28, 52, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, 189) were measured by high resolution gas chromatography with electron capture detection by the National Reference Center for Dioxins and Related Compounds, in Bratislava, Slovakia in 1999, which has been certified by World Health Organization (WHO) for the analysis of PCBs in human tissues [2, 6].

Genotyping of three functional polymorphisms in the glutathion-S-transferase (GST) genes *GSTT1* and *GSTM1* (deletion), *GSTP1* (point mutation, Ile-105-Val, changed affinity of substrate to the enzyme, changed kinetics) were conducted in 2002 by the Department of Experimental and Applied Genetics of the Institute of Preventive and Clinical Medicine in Bratislava. Standard analytical methods were used which are in detail described elsewhere and referenced here [7-9].

Results and Discussion

Geometric mean level of the sum of 15 PCB congeners in the whole sample was 3,200 ng/g of lipid. In 66 persons living within 10 km range from the PCB production facility, the level was 4,205 ng/g of lipid. Levels in subjects more than 10 km from the plant, further downstream, and south and east from the plant, and around the water reservoir contaminated with PCBs, were lower, 2,634 ng/g, but still almost three times higher than in non-PCB contaminated district of eastern Slovakia (Svidnik, approximately 950 ng/g lipid). Table 1 also shows similarities in basic

Table 1. Basic characteristics of study participants and distribution of genotypes in two exposure groups in the Michalovce district.

Variable		<10km from PCB plant	≥10 km from PCB plant
Age (years) [a]		46.5	49
Body Mass Index (kg/m^2) [a]		26.9	26.7
Education (>8 years)		35 (53%)	75 (56%)
Consumes locally grown food Yes (%)		33 (50%)	93 (69%)
Alcohol consumption	0	13 (20%)	28 (20%)
(drinks/week)	1-2	29 (44%)	53 (40%)
	≥ 3	24 (36%)	53 (40%)
Smoking status	Never	37 (56%)	80 (60%)
	Former	18 (27%)	27 (20%)
	Current	11 (17%)	27 (20%)
Group 1 PCB [b]		68 (23-1,266)	58 (9-824)
Group 2 PCB		444 (101-5,486)	309 (62-5,870)[§]
Group 3 PCB		3,623 (1,016-27,492)	2,241 (231-53,792) [§]
Sum PCB		4,205 (1,143-30,341)	2,634 (353-60,486) [§]
DDT		95 (30-710)	121 (4-909)
DDE		2,913 (588-14,513)	2,977 (119-19,912)
HCB		1,252 (55-7,815)	1,307 (109-8,504)
GSTT1 Deletion		79 (59%)	35 (53%)
Gene Present		55 (41%)	31 (47%)
GSTM1 Deletion		43 (32%)	21 (32%)
Gene Present		91 (68%)	45 (68%)
GSTP1 aa (wild type)		71 (53%)	27 (41%)
ab		51 (38%)	36 (54%)
bb		12 (9%)	3 (5%)

[a] Median; [b] All organochlorines levels are age adjusted geometric means, range in the brackets.
[§] Statistically significant at α=0.05

demographic and lifestyle characteristics, and the distribution of polymorphism in these two groups. No association with the variant genotypes of three analyzed enzymes were observed in the whole sample. Variant genotypes in GST family did not seem to influence the levels of the sum of PCBs, individual PCB congeners, DDT/DDE, HCH or HCB (data not shown). These results are consistent with previous results in some PCB-breast cancer studies [11-12].

In the highest exposed group, 66 persons living within 10 km of the former production facility, we observed statistically significant positive association with *GSTT1*(-) (sum PCBs: 5,579 *GSTT1*(-) versus 3,525 ng/g *GSTT1*(+), p=0.02). Similar associations were observed for estrogenic (group1), mono-ortho (group2) and di-ortho (group 3) PCB congeners (Table 2). In contrast, PCB levels were higher when *GSTM1* gene was present (sum PCBs: 3,730 versus 4,633 ng/g, p=0.21) but only for mono-ortho PCBs did this association reached statisticalsignificance (p=0.05). No consistent pattern was seen for *GSTP1* or for other organochlorines. No confounding effect of smoking or alcohol consumption was observed in these analyses. Results of our study suggest that some persons may have higher levels of PCBs partly due to their genetic makeup and consequently different metabolism of these compounds. Analyses of other enzymes involved in biotransformation of organochlorines are needed to better understand the interactions between exposure and metabolism of these compounds.

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Table 2. Polymorphisms in glutathion S-transferase enzymes and age adjusted levels of organochlorines associated with different genotypes in persons living within 10 km of former PCB production plant (ng/g of lipid).

	N	Sum PCB	p-value [d]	Group 1 [a]	p-value	Group 2 [b]	p-value	Group 3 [c]	
GSTT1									
Deletion	35 (53%)	5,579		252		643		4,670	
Gene Present	31 (47%)	3,525	0.015	58.5	0.03	351	0.004	3,082	0.027
GSTM1									
Deletion	21 (32%)	3,730		61.3		361		3,289	
Gene Present	45 (68%)	4,633	0.21	76.2	0.26	534	0.047	3,893	0.33
GSTP1 (Ile105Val)									
aa (wild type)	27 (41%)	4,056		64.0		403		3,564	
ab	36 (54%)	4,429	0.62	73.9	0.47	487	0.35	3,784	0.77
bb	3 (5%)	2,091	0.13	41.1	0.35	209	0.18	1,841	0.12

[a] PCB congeners 28, 52, 101; [b] PCB congeners 105, 114, 118, 123, 156, 157, 167, 189; [c] PCB congeners 138, 153, 170, 180.
[d] p-value from the analyses of covariance comparing age adjusted geometric means of organochlorine concentration.