Effects of dioxins, PCBs and PBDEs on immunology and haematology in adolescents

Marike M. Leijs[1,2], Janna G. Koppe[3] , Kees Olie[2], Wim M.C. van Aalderen[1], Pim de Voogt[2,4], Gavin W. ten Tusscher[5]

[1] Department of Paediatrics and Neonatology, Emma Children's Hospital Academic Medical Centre, Amsterdam, Netherlands.

[2] IBED/ESS, University of Amsterdam, Netherlands.

[3] Ecobaby Foundation, Loenersloot, Netherlands.

[4] KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB Nieuwegein, Netherlands

[5] Department of Paediatrics and Neonatology, Westfriesgasthuis, Maelsonstraat 3, 1624 NP Hoorn, Netherlands

ABSTRACT

Dioxins and PCBs are environmental pollutants, proven to be immunotoxic. In this longitudinal cohort, at birth and at follow-up during childhood, immunological and haematological effects were seen, prompting us to perform a new follow-up during adolescence.
Methods:
Vena puncture was performed to assess haematological (Haemoglobin, thrombocytes, thrombopoietin) and immunological (leukocytes, leukocyte differentiation) parameters and the current serum levels of dioxin, dl-PCBs and PBDEs.
Results:
A decrease in the number of polymorphic neutrophils was found in adolescents with higher dl-PCBs in their serum ($p=0.021$). No relationship with leukocyte counts, thrombocytes, haemoglobin or thrombopoietin levels was seen. Similarly, we found no relationship between prenatal, nor current, dioxin levels and the hematological and the immunological parameters determined.
The $\sum$PBDEs were negatively associated with the number of lymphocytes ($p=0.01$) and positively with the haemoglobin concentration ($p=0.003$).
Conclusion:
These effects on the innate immunity by current levels of dl-PCBs and on the adaptive immunity by PBDEs are disconcerting, especially as the dl-PCB (0.04-7.8 WHOTEQ pg/g lipid, mean: 2.2 WHOTEQ pg/g lipid) and $\sum$PBDE levels (mean 14.04 ng/g lipid, including one outlier with a sum of 73.6 ng/g lipid) were not exceptionally high.

INTRODUCTION

Dioxins (PCDD/F), and especially 2,3,7,8-tetrachloro-dibenzo-ρ-dioxin are well known immunotoxicants. Effects on the cell-mediated and humoral-mediated immunity have been seen in animals [1]. An important target organ of dioxin immunotoxicity is the thymus, where maturation of T-lymphocytes takes place. Atrophy and suppression of the thymus-dependent immunity have been noted [2]. Administration of dioxins during the perinatal period has proven to cause severe immunosuppression in rats and mice [3].

Pluim et al, in the same group of children as the current cohort, found a reduced number of polymorphic neutrophils in relation to prenatal and lactational dioxin exposure; in the same study thrombocyte counts appeared to be significantly decreased, in relation to increasing lactational dioxin exposure [4]. The pronounced effect on the number of polymorphic neutrophils around birth, an effect on the innate immunity, was confirmed in another study in Rotterdam [5]. However, these effects were no longer evident at the 30-month follow-up [6].

Follow-up of our cohort during childhood revealed a persistently decreased number of thrombocytes, with a significant increase in thrombopoietin, as well as a possible decrease in allergy. We found the outcomes suggestive of a toxic effect at stem cell level. The lymphocyte counts showed an increased $CD4^+$ T-helper and increased CD45RA number in relation to perinatal PCDD/F concentration at prepubertal age [7].

MATERIALS AND METHODS

Study population:

This study is part of a longitudinal cohort study of currently 14-19 year old children, studied during their neonatal (n=60) [8], toddler (n=60) [9] and pre-pubertal period (n=41) [10]. All 33 children (18 girls and 15 boys) participating in the current follow-up were born in the Amsterdam/Zaandam area. PCDD/F exposure was determined shortly after birth in the breast milk of their mothers. The study was approved by the institutional medical ethics committee. All participants of the study and their parents signed an informed consent.

Laboratory analyses

For measurements of the current PCDD/F, dl-PCB and PBDE serum concentrations, 30 subjects underwent vena puncture, following a fasting period of at least four hours. Perinatal PCDD/F levels and current serum levels of PCDD/Fs, dl-PCBs and PBDEs were measured in a contamination-free laboratory at the Department of Environmental Chemistry of the University of Amsterdam. Concentrations of the 19 most toxic dioxin congeners (seven PCDDs and twelve PCDFs) and the concentration of 3 dl-PCBs (#77, 126, 169) and 8 PBDEs (#28, 47, 85, 99, 100, 153, 154 and 183) were determined. The concentrations of PCDD/Fs and dl-PCB congeners were expressed in TEQ pg/g lipid using the new WHO TEF values [11]. The prenatal and lactational TEQ values are expressed in International Toxic Equivalents (I-TEQ) as used in 1991.

Levels of leptin, glucose, insulin, lipid spectrum, immunological parameters, haematological parameters and thyroid parameters were measured in the serum of the adolescents.

Statistical analyses

For statistical analyses the non-parametric Spearman's correlation coefficient was calculated using the software package SPSS. The level of significance was 5%. One outlier in the level of sumBDE was excluded from the statistical analysis.

RESULTS

Haematology:

The age of the cohort and the amount of perinatal and current dioxin exposure, as well as the current dl-PCB and PBDE levels in serum, are shown in table 1.

Serum levels of haemoglobin, the number of blood platelets and levels of trombopoietin are presented in table 2.

Using the non-parametric Spearman's correlation coefficient, we found a positive relationship with the haemoglobin level and the current serum PBDE concentration (figure 1; p=0.003). Congener specific analysis of the sum of PBDEs showed that the main contributors to this relation were PBDE congeners 85 (p=0.032) and 153 (p=0.066). No associations with PCDD/Fs or dl-PCBs were evident.

Contrary to the findings during infancy and at the age of 8-12 years, no association was seen between the current thrombocyte count, nor with current thrombopoietin levels, and the perinatal and current dioxin, dl-PCB or PBDE exposure.

Immunology

Effects on the immune system were evaluated using the leukocyte and differentiation counts. Mean values and ranges of the outcomes are shown in table 2.

A negative effect on polymorphic neutrophils was found in adolescents with higher current dl-PCB levels in their serum (figure 2; p=0.021). In the neonatal period this negative effect was seen with prenatal PCDD/F exposure. No relationship between neutrophils and perinatal or current dioxins or PBDE levels in serum was seen.

The PBDEs showed a negative relationship with the number of lymphocytes (figure 3; p=0.01). Congener specific analysis of the sum PBDEs showed that the main contributors to this association were PBDE congeners 183 (p=0.008), 154 (p=0.009) and 85 (p=0.03).

DISCUSSION

Haematology

We found a positive relationship between serum haemoglobin (Hb) content and the serum ∑PBDEs (p=0.003). Erythrocytes and thrombocytes have, during maturation, a common pathway of precursors, before they differentiate into erythroblasts and megakaryocytes. The differentiation towards erythroblast production can be influenced by delta-aminolaevulinic acid [12]. An increase in delta-aminolaevulinic acid is found after PCB, polybrominated biphenyl (PBB) and lead intoxication [13]. This may be a mechanistic explanation of our findings with PBDEs. Another possible explanation is a higher production of erythropoietin in the kidney [14].

Immunology

Human immunity can be divided into the innate immune system and the adaptive immune system. The latter is highly specific to invading micro-organisms and has a memory function. The innate immunity contributes to host defense immediately after infection, before the adaptive immunity becomes active. Natural killer cells, monocytes, macrophages and polymorphic neutrophils form the innate immunity.

In our study a negative effect was found on the number of polymorphic neutrophils (p=0.021) in relation to dl-PCBs, similar to the dioxin effect seen in the neonatal period. PCBs and some of the BFRs induce formation of ROS (Reactive Oxigen Species) in neutrophil granulocytes, in animal studies. This has an adverse effect on the neutrophil granulocytes [15].

Generally, dioxin effects on the immune system are dependent on signaling through an intracellular receptor known as aryl hydrocarbon receptor (AhR) [16]. Many of the specific effects of PCDD/Fs and dl-PCBs on the immune system are also dependent on the modulation that the active form of the AhR can exert on the NF-□B signaling pathway, and the regulation of genes that code for growth factors, cytokines, and pro-apoptotic and anti-apoptotic factors [17,18]. The NF-□B signaling pathway is complex, and immune system functions depend on a tight regulation of cellular proliferation, growth, survival, and apoptosis in many physiological situations, like ontogenic development, adaptive cellular and humoral immune response, inflammation, and innate immune responses [19,20].

In our cohort, when studied at prepubertal age (8-12 years), we found abnormalities in T- lymphocytes in relation to prenatal PCCD/F exposure (increase in CD4 +). Similarly, the Rotterdam preschool study showed the number of T-cells to be increased, together with lower antibody levels against measles and a higher prevalence of chickenpox at 18 months, with prenatal dioxin TEQ, while at 42 months the relation was with ∑PCBs. Both PCBs and dioxins are known to change the kinetics of thymocyte maturation and thymus atrophy in in vitro studies. In Japanese breast-fed infants the $CD4^+$, $CD8^+$ and ratio between the two lymphocyte subsets was shown to be positively influenced by the amount of dioxins consumed [21].

A study of progenitor cells (PC) exposed to a single dose of the PCB mixture Aroclor 1248 showed the production of a factor(s) that activates neutrophils, stimulating them to damage PC populations in culture [22].

We also found a negative association between the lymphocyte counts and PBDEs (p=0.01).

The toxicity of some PBDE congeners may be compared with phenobarbital. This anti-epileptic medicine may lower lymphocytes counts [23]. An in vitro study in human lymphocytes, however, showed no effects of PBDEs on the proliferation and immunoglobulin synthesis [24].

A Chinese study demonstrated negative effects on the T-lymphocytes, in the offspring of PBDE-209 exposed rat.

Conclusion:

A lower number of polymorphic neutrophils was seen in our cohort, during the neonatal period with PCDD/F exposure and in puberty with dl-PCB exposure. PBDEs have a negative effect on the number of lymphocytes. It is alarming that not only the rather high dioxin and PCB levels in the neonatal period, but also the current levels of dl-PCBs and PBDEs have persistent and/or recent effects on respectively the innate and adaptive immunity.

ACKNOWLEDGMENTS

The authors wish to thank: the participants of the study, and their parents, for their time, enthusiasm and cooperation; Dr. J. Oosting for his help with the statistics; and P. Serné, F. vd Wielen and J. Parsons of the laboratory of the Institute for Biodiversity and Ecosystem Dynamics and T. van Teunenbroek of the Netherlands Ministry of Housing, Spatial Planning and the Environment. We are indebted to Matthijs Westra, M.D. and the Zaans Medical Centre for their help in performing the study.

The paper was supported by E.C. grant: HENVINET 037019

Table 1: Mean age, BMI and dioxin, dl-PCB and PBDE exposures

	Mean	Range
Age (years) n=30	15.0	14.0-18.7
Prenatal PCDD/F exposure ITEQ (pg/g lipid)	32.6	9.05-88.8
Lactational PCDD/F exposure ITEQ (ng)	66.9	4.34-279
Current serum PCDD/F WHOTEQ (pg/g lipid)	2.2	0.4-6.1
Current serum dl-PCBs WHOTEQ (pg/g lipid)	2.2	0.04-7.8
Current serum PBDE (ng/g lipid) n=18	13.9	4.9-73.6

Table 2: Serum/blood parameters: mean values and ranges observed

	Mean	Range
Thrombocytes (10^9/l)	268	156-352
Haemoglobin (mmol/l)	8.47	6.70-9.70
Thrombopoietin (E/ml)	18.9	6.00-41.0
HbA1C (%)	5.60	5.00-6.10
Leukocytes (10^9/l)	6.94	3.40-10.4
Polymorphic neutrophils (10^9/l)	4.03	1.71-7.49
Lymphocytes (10^9/l)	2.12	1.04-3.44
Monocytes (10^9/l)	0.49	0.32-0.86
Eosinophils (10^9/l)	0.25	0.05-0.69
Basophils (10^9/l)	0.04	0.00-0.21

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