CHANGES OF BIOLOGICAL PARAMETERS IN BREAST-FED INFANTS DUE TO PCDD/PCDF/PCB BACKGROUND EXPOSURE ?

Klaus Abraham, Olaf Päpke*, Ulrich Wahn and Hans Helge

Children's Hospital, University Clinic Charité, Humboldt University Berlin,
Augustenburger Platz 1, 13 353 Berlin, Germany
*ERGO Forschungsgesellschaft, Geierstr. 1, 22 305 Hamburg, Germany

Introduction

The accumulation of lipophilic and biologically persistent chlorinated hydrocarbons in the food chain leads to a relatively high exposure of breast-fed infants. Compared to their mothers, clearly higher PCDD/F concentrations were measured in 11-month-old infants following six to seven months of full breast-feeding (1). The fact that infants breast-fed for several months reach relatively high PCDD/PCDF/PCB concentrations compared to general background levels has caused concern about possible negative health effects in these children, whose developing biological functions (e.g. immune system) might be more sensitive. On the other hand, breast-feeding has several advantages for mother and child, and is recommended by paediatricians world-wide. A few investigations were performed in recent years dealing with the question of measurable changes of sensitive biological parameters in breast-fed infants due to the PCDD/PCDF/PCB background exposure. For the immune system, decreasing CD4/CD8 ratios with increasing PCB concentrations in mother's milk were reported (2) as well as increasing ratios associated with calculated total I-TEq intake (3). For thyroid function, TSH serum concentrations were reported to increase with increasing PCDD/PCDF/PCB exposure (4,5).

Study design

We performed a new study in 11-month-old infants who had been fully breast-fed for at least four months. The main aim was to measure an extensive test program for biological parameters possibly influenced by the PCDD/PCDF/PCB exposure. In contrast to other studies, this exposure could be directly determined by measurement of the concentrations in blood fat. We especially looked for infants in whom different levels of exposure had to be expected: lower exposure (young mother, siblings also breast-fed for a longer time), or higher exposure (older mother, first infant breast-fed, region with higher PCDD/PCDF background contamination). Children had to meet the following inclusion/exclusion criteria: healthy infants (German by descent in order to reduce genetic variability and cultural influences) born at term, no new infection during the last two weeks before blood sampling, tetanus and diphtheria vaccination at least twice, no passive exposure to cigarette smoke. Additionally, we looked for formula-fed infants (breast-fed for less than 2 weeks) with expected very low PCDD/PCDF/PCB exposure.

Material and Methods

Heparin blood was taken by the same person in the morning before breakfast from the infant (15 ml) and the mother (40 ml) who was also interviewed for anamnestic data e.g. regarding her possible PCDD/PCDF/PCB exposure and child's medical history during pregnancy, birth and the first year of life. The blood had to be transferred to the laboratory within 4 hours. From infants

blood, whole blood was taken for blood count, lymphocyte subpopulations (three colour FACS analysis using MABs), lymphocyte proliferation (^{3}H-thymidin incorporation following stimulation of diluted whole blood with mitogens and antigens) and heavy metals (possible confounder). After centrifugation, plasma was analysed for routine clinical chemistry including immunoglobulins. A part of the plasma was frozen at –80^{o}C for analysis of thyroid function, specific antibodies (tetanus and diphtheria toxoid, HiB), IgG subclasses, IgE and other parameters to be performed at the end of the sample collection period. The remaining plasma (3 to 5 ml) as well as the maternal plasma (about 18 ml) was frozen at –20^{o}C for analysis of PCDDs, PCDFs, PCBs, DDE, HCB and γ-HCH. Measurements were performed at the ERGO Forschungsgesellschaft, as described previously (6). Remaining red cell mass including white cells was used for ficoll and subsequent percoll separation in order to isolate lymhocytes/monocytes (peripheral blood mononuclear cells, PBMCs) and granulocytes, respectively. PBMCs were used to detect cytokine production following stimulation with phytohemagglutinin (PHA). Remaining cells were cryoconserved and stored in the vapour phase of liquid nitrogen, for later quantitative CYP (cytochrome P450) 1A1 mRNA measurements using competitive RT-PCR. Granulocytes were used for measurement of chemiluminescence.

Results and Discussion

Until May 1999, 100 children had been investigated. Of the 79 breast-fed infants, 27 were from the region Ilsenburg/Harz with higher average PCDD/PCDF contamination of mother's milk due to a copper recycling plant closed 1990 (7). PCDD/PCDF analysis completed for 74 children showed I-TEq concentrations (Nato TEFs, PCBs not included, based on blood fat calculated from cholesterol and triglycerides (8)) between 8.1 and 107 ppt (median 25.3 ppt). Of these children 6 had I-TEq concentrations higher than 50 ppt, five of them came from the region Ilsenburg. From the 21 formula-fed infants, individual PCDD/PCDF analysis was performed in five children. I-TEq concentrations were found between 1.9 and 3.2 ppt. For the biological parameters evaluated until May 1999 (blood count, clinical chemistry, immunoglobulins, lymphocyte proliferation following stimulation with tetanus and diphtheria toxoid, main lymphocyte subpopulation including CD4/CD8 ratio), no significant association with I-TEq concentrations in blood fat was observed.

Acknowledgements

This investigation was supported by grants from Forschungszentrum Karlsruhe, Baden-Württemberg (PUG U 96 005).

References

1. Abraham K, Knoll A, Ende M, Päpke O, Helge H *Pediatic Research* **1996**, 40, 671-679
2. Dewailly E, Bruneau S, Laliberte C, Belles-Iles M, Weber JP, Ayotte P, Roy R *Organohalogen Compounds* **1993**, 13, 403–406
3. Nagayama J, Tsuji H, Iida T, Hirakawa H, Matsueda T, Okamura K, Hasegawa M, Sato K, Ma HY, Yanagawa T, Igarashi H, Fukushige J, Watanabe T *Chemosphere* **1998,** 37, 1781-1787
4. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LGMT, Brouwer A, Sauer PJJ *Pediatic Research* **1994**, 36, 468-473
5. Nagayama J, Okamura K, Iida T, Hirakawa H, Matsueda T, Tsuji H, Hasegawa M, Sato K, Ma HY, Yanagawa T, Igarashi H, Fukushige J, Watanabe T *Chemosphere* **1998,** 37, 1789-1793
6. Päpke O *Organohalogen Compounds* **1997**, 31, 212-214
7. Abraham K, Palavinskas R, Wahn U, Helge H, Mathar W *Organohalogen Compounds* **1998**, 38, 33-36
8. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham LL *Archives of Environmental Contamination and Toxicology* **1989**, 18, 495-500