RELATIVE POTENCY ESTIMATES OF DIOXIN-LIKE ACTIVITY FOR DIOXINS AND FURANS IN HUMAN ADULTS USING TWO THYROID OUTCOMES

Trnovec T[1]*, Wimmerová S[1], Šovčíkova E[1], Lancz K[1], Chovancová J[1], Patayová H[1], Palkovičová Ľ[1], Drobná B[1], Langer P[2], Van den Berg M[3]

[1]Slovak Medical University, Limbová 12, 83303 Bratislava, Slovakia; [2]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia; [3]Institute for Risk Assessment Sciences – IRAS, Utrecht University, Tijdelijke huisvesting Jenalaan 18D, 3584 CK Utrecht, The Netherlands

Introduction

For the risk assessment of PCDD, PCDF and DL-PCB congeners have been assigned a toxic equivalency factor (TEF) value relative to the toxicity of TCDD by the World Health Organization (1). The re-evaluation of TEF values for these compounds has become a continuous process based on new available scientific information for which results from *in vivo* as well as *in vitro* studies have been used. Although many studies with human cell-lines or primary cells have been published to date (2), the availability of human *in vivo* data that may contribute to the TEF concept has not been published until now.

We re-analyzed data on thyroid impairment (3) in a population exposed to a mixture of organochlorines and tried to identify toxicity of individual mixture components. By using the outcome of this re-analysis we tried to estimate the relative toxic potencies (REPs) of PCDD, PCDF and DL-PCB congeners in adult humans based on two thyroid outcomes, the thyroid volume and free thyroxin (FT4) serum concentration.

Materials and methods

In the past we have determined the exposure to dioxin-like compounds (DLCs) and health status of a population living in the towns and villages of the Michalovce, Svidnik and Stropkov districts in eastern Slovakia, an area contaminated by a complex cocktail of organochlorines (4-6). The inclusion criterion of all subjects into the study was a long-term permanent residence in the study area. The study protocol was approved by Institutional review board at the Slovak Medical University in Bratislava. This cross-sectional epidemiological study consisted of two parts: The basic part involved physical examination of 2047 adults, 51% female and 49% male. Individual blood samples were collected and analyzed for PCBs and organochlorine pesticides and hormones. In addition in a subgroup of 320 adults, we analyzed PCDDs, PCDFs, and some dioxin-like (DL-)PCBs. The analytes were quantified by high-resolution GC-MS (MAT 95XL, Germany). The thyroid volume was assessed by sonography (7).

REPs of individual compounds of the dioxin-like mixture, to which the population of eastern Slovakia was exposed, were estimated by comparison of the toxicity of its individual members with that of TCDD. Two health outcomes were evaluated: thyroid volume and serum concentration of FT4. Two approaches were used for estimation of the relative potencies of individual components of the mixture. The first, suggested previously (8, 9) was based on a comparison of benchmark concentrations (BMCs) calculated for thyroid outcomes vs. organochlorine serum concentrations. The other is based on comparison of the regression coefficient (β) between thyroid volume or FT4 serum concentration and serum concentration of the individual congeners, in an analogous way as described earlier (10). The BMCs for changes of thyroid volume and serum FT4 associated with TCDD concentration were compared with the BMCs of individual congeners and were considered to be the congener specific REPs. Thus BMC(1)/BMC(i) is a relative potency (REP(i)) indicating relative toxicity of the i-th congener compared to the TCDD. Using the regression approach, REPs of the individual congeners were calculated as the ratio of the slope β obtained for the ith congener to the slope β for TCDD, as β_i/β_{TCDD}. The REPs resulting from both approaches were compared with published data on REPs for DLCs (2) and with the published WHO-TEF values (1).

Results and discussion

The PCDD congeners were associated with a decrease in both thyroid volume and FT4 level, except in the case of 1,2,3,7,8,9-HxCDD for thyroid volume and OCDD for FT4 concentration. The PCDFs though, depending on the congener, were associated with both an increase and a decrease in thyroid volume and FT4 level, while the DL-PCBs were quite uniformly related to an increase in the thyroid volume and FT4 serum level, except in the case of PCB 81 for thyroid volume and PCB 105 for FT4. TCDD was most strongly associated with a decrease of thyroid volume and FT4 level. To comply with the assumption of a similar mode of action of compared congeners (11) and associated additivity, we calculated only REPs for those acting in the same direction as the index chemical TCDD.

We also evaluated the confounding effect of age and gender of our subjects and other DLCs present in serum using multiple regression analysis. Our results indicate that BMCs and benchmark concentration lower confidence limits (BMCLs) for TCDD are slightly influenced by the presence of other congeners in the exposure mixture.

REPs calculated using the BMC, BMCL and regression slope data correlated significantly between themselves (all correlation coefficient values >0.903 and p<0.001). Importantly, REPs calculated from the thyroid volume data correlated with those from the independent FT4 data. Statistically significant correlation was found between REPs for individual congeners derived from thyroid volume and FT4 data using β_i/β_{TCDD} (R=0.81 and p=0.015), BMCL (R=0.857 and p=0.007) and BMC (R=0.786 and p=0.021) approach. From calculated REPs correlated significantly (R value=0.622 and p=0.031) with the WHO TEF values (1) the regression slope derived data.

The correlation between the logarithm of our REPs for thyroid volume and TEFs can be described by equation:

Log REP = [0.615± 0.192(SE)] x LogTEF - 0.187 (p for slope=0.009).

The relationship between FT4 and TEFs can be described by the equation:

Log REP = [0.109± 0.206(SE)] x LogTEF - 0.673 (p for slope=0.612).

The outlying REP for OCDF obviously decreases the latter correlation. The REPs for all PCDD congeners studied and thyroid volume, irrespective of the method of derivation, are between the maximum and minimum values estimated by other researchers (2). TheREPs for FT4 that were associated with the dioxins congeners 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD and 1,2,3,4,6,7,8-HpCDD were higher than the published maximum estimates (2).

For PCDF congeners associated with a decrease of thyroid volume the REPs are rather close to the maximum values determined by other investigators, except for 2,3,4,7,8-PeCDF that is still above the minimum reported value of 0.0065 (2). For the relationship between PCDFs and FT4, we calculated the REPs for four congeners from which those of 2,3,7,8-TCDF and 1,2,3,4,6,7,8-HpCDF are in the upper region of published estimates, while OCDF behaved like an outlier with regard to REP value..

With regard to our study design, several issues have to be considered. First is the selection of endpoints for exposure-effect analysis. We have chosen thyroid endpoints because of the broad knowledge on thyroid pathology in animals and humans exposed to organochlorines (12-16), including that from our own studies (3, 17). Moreover, a decrease of thyroid hormone T4 has been suggested as a prospective biomarker for generating a new human TEF scheme (9). In addition, the many reported inconsistencies in findings on thyroid health outcomes in subjects environmentally exposed to DLCs (9, 18) also present a challenge with respect to the interpretation of our results.

A significant finding in the present study is that the exposure to the index chemical, TCDD, and to several components of the DLCs mixture in blood was associated with a simultaneous decrease in thyroid volume and FT4 serum concentrations. The REPs currently calculated by two completely different approaches, one type based on thyroid morphology and the other on thyroid hormonal endpoint, agree unexpectedly well. This agreement provides more support for the observed REPs by us. In spite of different designs used in various published REP studies compared to our study, most calculated REPs e.g. for dioxins and thuyroid volume ,fit well within the ranges of published REPs (2).

The strengths of our study are:

1. It is based on two types of human thyroid endpoints with an apparent completely different pathogenesis.,as the homeostasis of FT4 is probably not directly related to thyroid size.
2. We have evaluated serum concentrations of individual DLCs that reliably reflect the human systemic body burden. Thus our exposure data may be more suitable for comparison of relevant health endpoints than the intake data used in many experimental and human studies.

However, a number of weaknesses of our study can also be observed. These are:

1. The exposure scenario under laboratory conditions takes into account one single chemical while under field conditions we are usually facing exposure to a complex mixture of chemicals with different potencies and often not even the same mode of action.
2. Single exposure data does not necessarily reflect the whole life time exposure of each subject.

Acknowledgements

This work was supported by the EU 5th Framework Programme project "Evaluating human health risk from low-dose and long-term PCB exposure" QLK4-CT-2000-00488 (PCBRISK) and EU 7th Framework Programme project No: 226694 "The development, validation and implementation of human systemic Toxic Equivalencies (TEQs) as biomarkers for DLCs".

References

1. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. (2006) *Toxicol Sci.* **93:** 223-241

2. Haws LC, Su SH, Harris M, Devito MJ, Walker NJ., Farland WH, Finley B, Birnbaum LS. (2006) *Toxicol Sci.* **89:** 4-30
3. Langer P. (2010) *Front Neuroendocrinol.* **31:** 497-518
4. Jursa S, Chovancová J, Petrík J, Lokša J. (2006) *Chemosphere* **64:** 686-691
5. Langer P, Kocan A, Tajtáková M, Rádiková Z, Petrík ., Koska J, Ksinantová L, Imrich R, Hucková M, Chovancová J, Drobná B, Jursa S, Bergman A, Athanasiadou M, Hovander L., Gasperíková D, Trnovec T, Seböková E, Klimes I. (2007) *Chemosphere* **70:** 110-118
6. Petrik J, Drobna B, Pavuk M, Jursa S, Wimmerova S, Chovancova J. (2006) *Chemosphere* **65,** 410-418
7. Brunn J., Block U, Ruf G., Bos I, Kunze WP, Scriba PC. (1981) *Dtsch Med Wochenschr*. **106:** 1338-1340
8. Fattore E, Chu I, Sand S, Fanelli R, Falk-Filippson A, Håkansson H. (2004) *Organohalogen Compounds* **66:** 3402-3407
9. Yang JM., Salmon AG, Mart MA. (2010) *Regul Toxicol Pharmacol* **56:** 225-236
10. Brown DJ, Chu M, Van Overmeire I., Chu A, Clark GC. (2001) *Organohalogen Compounds* **53:** 211–214
11. EPA/630/R-00/002, August 2000 Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533
12. Boas M, Main KM, Feldt-Rasmussen U. (2009) *Cur Opin Endocrinol Diabetes Obes.* **16:** 385-391
13. Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, Carchman RA, Carter WH. Jr, DeVito MJ. (2005) *Enviro. Health Perspect.* **113:** 1549-1554
14. Zoeller TR. (2010) *Hormones* **9:** 28-40.
15. Zoeller TR, Dowling AL, Herzig CT, Iannacone EA, Gauger KJ, Bansal R. (2002) *Environ Health Perspect*. **110**: Suppl 3**,** 355-361
16. Zoeller RT. (2007) *Thyroid* **17:** 811-817
17. Rádiková Z, Tajtáková M, Kocan A, Trnovec T, Seböková E, Klimes I, Langer P. (2008) *Thyroid* **18:** 353-362
18. Goodman JE, Kerper, LE, Boyce CP, Prueitt RL, Rhomberg LR. (2010) *Regul Toxicol Pharmacol.* **58:** 79-99