EVIDENCE FOR ESTROGENIC AND TCDD-LIKE ACTIVITIES IN EXTRACTS OF BLOOD AND SEMEN OF MEN

Miroslav Machala, Jan Vondrácek*, Robert Ulrich, Jiří Rubeš, Radim Šrám**

Veterinary Research Institute, 621 32 Brno, Czech Republic
* Institute of Biophysics, Academy of Sciences of the Czech Republic, 612 65 Brno, Czech Republic
**Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic

Introduction

Epidemiological studies suggest that reproductive impairment can be associated with environmental chemical contamination. Recently, significant effects of environmental pollution on reproductive outcomes including impaired semen quality in the human population living in a Czech brown coal mining area have been described [1]. In this paper, organic extracts of blood and semen of young men collected in this region, were examined for estrogen and Ah receptor-mediated activities using *in vitro* reporter gene expression assays. The aim of the study was to reveal the presence of xenoestrogens and dioxin-like compounds capable of causing reproductive disorders. The concentrations of polychlorinated biphenyls, organochlorine contaminants and nonylphenol were also determined in the extracts.

Materials and Methods

Samples of ejaculate and whole blood were collected from 25 young men from the North Bohemian region, Czech Republic. The ejaculate samples were extracted on a solid phase extraction column SPE 18 with chloroform, evaporated and dissolved in isooctane. The whole blood samples were extracted 2 hours on a Soxtec Tecator apparatus with petroleum ether/acetone (1:1, v/v), purified on a Florisil column, evaporated and dissolved in isooctane.

In vitro assays of estrogenic and dioxin-like activities were performed with the MVLN cells and H4IIE cells, permanently transfected with a luciferase reporter gene under control of estrogen or dioxin responsive enhancers, respectively [2, 3]. The cells were kindly supplied by Dr. A.J. Murk, Wageningen Agricultural University, The Netherlands and Dr. Michel Pons, Montpellier, France.

The concentrations of PCBs and organochlorine contaminants were determined in aliquots of the extracts by a GC/ECD method using a DB-5 and DB-1705 double column system. The presence of 4-nonylphenol and 17β-estradiol was determined in selected extracts by GC/MSD and ELISA, respectively.

Results and Discussion

The sample extracts were screened for *in vitro* estrogenic and dioxin-like activities using transgenic cell lines (Fig. 1). A weak dioxin-like activity was detected in a majority of ejaculate and blood samples and a relatively high estrogenic activity was found in several samples of semen and blood (note the different dilutions used in the bioassays).

Chromatographic analyses of the extracts showed that PCBs, o,p'-DDE, p,p'-DDE, and lindane ranked with the most important environmental contaminants found in the samples (data not shown). The concentrations of PCBs in ejaculate and blood were in individual subjects similar ranging between 1 and 12 ng/ml. While low concentrations of hexachlorobenzene were detected, lindane, as the prevalent isomer of hexachlorocyclohexane, was found in relatively high concentrations of up to 21 ng/ml. Comparably high concentrations of DDT residues were determined in several samples. 4-Nonylphenol and 17β-estradiol, as other potential xenoestrogen and natural estrogen, respectively, were not detected in selected extracts showing the highest estrogenic activity.

Generally, the measurement of biochemical *in vitro* responses can complete data obtained by chromatographic analyses that may be insufficient owing to a limited number of the contaminant classes and possible antagonistic action of xenobiotics [4, 5]. Apparently, the estrogenic and dioxin-like activities detected in our study were not related only to higher concentrations of PCBs or organochlorine compounds in several samples; estrogenic and dioxin-like potentials can be attributed to other compounds for which the samples were not analysed.

In vitro assays, however, can be proposed as the initial step in risk assessment and evaluation of adverse effects on reproduction *in vivo*. A relatively high estrogenic potential of xenobiotics may represent one of the important mechanisms of reproductive impairment in men.

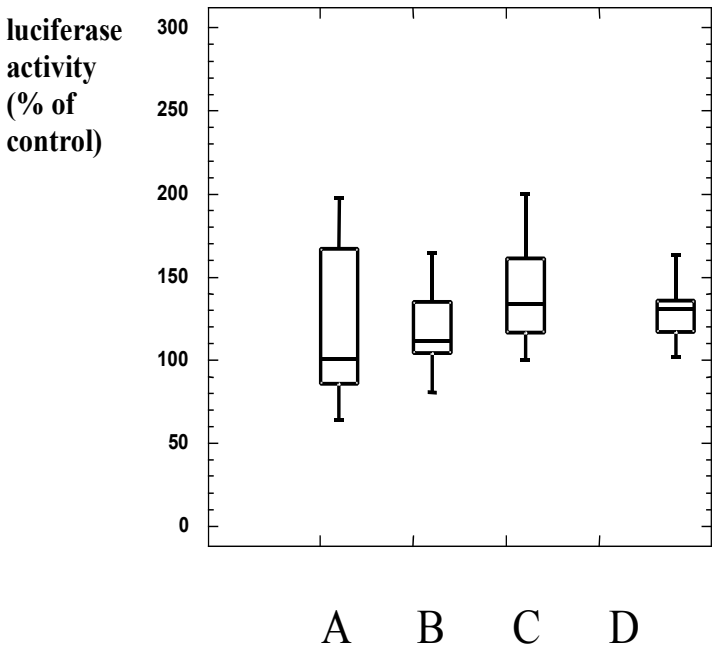

Figure 1: Results of analysis of estrogenic and TCDD-like activities detected in blood and human ejaculate samples shown as multiple Box-and-Whisker plot. The results are expressed as % of luciferase activity of control treated with solvent only. **A** - estrogenic activity of extracts of whole blood samples (an equivalent of 0.01 l of blood per 1 ml of medium was applied to the cells). **B** - estrogenic activity of extracts of human ejaculate (an equivalent of 0.002 l of semen plasma per 1 ml of medium was applied to the cells). **C** - TCDD-like activity of extracts of whole blood samples (an equivalent of 0.04 l of blood per 1 ml of medium was applied to the cells). **D** - TCDD-like activity of extracts of human ejaculate (an equivalent of 0.008 l of semen plasma per 1 ml of medium was applied to the cells).

Ackonowledgements
This project was supported by the Ministry of Environment of the Czech Republic (grant No. VaV/340/1/97).

References

1. Šrám R.J., Binková B., Perreault-Darney S., Rössner P., Rubeš J., Selevan S.G., Topinka J. and Dejmek J. *Mutation Res*., in print.
2. Pons M., Gagne D., Nicolas A.C. and Mehtali M., *Bio Techniques* **1990**, 9, 450.
3. Murk A.J., Leonards P.E.G., Bulder A.S., Jonas A.S., Rozemeijer M.J.C., Denison M., Koeman J.H. and Brouwer A. *Environ Toxicol. Chem*. **1997**, 16, 1583.
4. Gillesby B.E. and Zacharewski T.R. *Environ. Toxicol. Chem*. **1998**, 17, 3.
5. van der Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley M., Giesy J.P. *et al. Environ. Health Perspect*. **1998**, 106, 775.