HIGHLY MUTAGENIC PAHs OCCURING IN THE ENVIRONMENT ARE POTENT INDUCERS OF Ah RECEPTOR-MEDIATED RESPONSES IN VITRO

Miroslav Machala[1], Jan Vondráèek[1,3], Ludák Bláha[1,2], Kateøina Minksová[3], Miroslav Ciganek[1], Jiøí Neèa[1]

[1]Veterinary Research Institute, 62132 Brno, Czech Republic
[2]Department of Environmental Chemistry and Ecotoxicology, Faculty of Science, Masaryk University, 61137 Brno, Czech Republic
[3]Institute of Biophysics, Academy of Science of the Czech republic, 61235 Brno, Czech Republic

Introduction

A number of highly mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs) have been detected in various environmental samples, such as river sediments, airborne particles, and stable dust. Although their strong mutagenicity has been demonstrated in metabolically competent cells[1,2], epigenetic mechanisms contributing to their carcinogenic effects remain to be assessed. Dioxin-like toxicity mediated through activation of aryl hydrocarbon receptor (AhR) has been identified as an important mode of action of several PAHs[3-6]. However, no data concerning dioxin-like potencies of dibenzopyrenes, cyclopenta[c,d]pyrene, 7,12,-dimethylbenzanthracene, 5-methylchrysene, dibenzo[a,j]anthracene, and benzo[c]phenanthrene showing strong *in vitro* mutagenicity, are currently available. Thus, the objective of the study was to identify PAHs showing significant dioxin-like toxicity and present in relevant concentrations in environmental samples.

Materials and Methods

Analyses of PAHs. Concentrations of 16 US EPA priority PAHs and 9 additional PAHs were determined by chromatographic analyses using both HPLC and GC/MS. All samples were edxtracted, after freeze-drying, using Soxhlet extraction with dichloromethane for 2 h, followed by a clean-up on a GPC column.

CALUX assay. Rat hepatoma H4IIE cell line permanently transfected with luciferase reporter gene under the control of dioxin-responsive enhancers (pGudLuc1.1) was kindly provided by Dr. A.J. Murk (Wageningen University, The Netherlands). Cells were maintained and the CALUX assay was performed as described previously[7]. Briefly, cells, grown in 96-well plates, were exposed to selected PAHs for either 6 h or 24 h. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene were used as reference compounds. After exposure, the medium was removed and the cells were washed with PBS and lysed to release luciferase. The amount of luciferase was measured with a Luminoscan RS luminometer (Labsystems, Finland) using luciferin assay mix (Promega, USA). When appropriate, cytotoxicity was determined using MTT assay.

Results and Discussion

Dioxin-like activities of two groups of PAHs were determined in the chemical-activated luciferase gene expression (CALUX) assay. AhR-inducing potencies of nine mutagenic PAHs, including dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene, dibenzo[a,h]pyrene, cyclopenta[c,d]pyrene, 7,12,-dimethylbenzanthracene, 5-methylchrysene, dibenzo[a,j]anthracene, and benzo[c]phenanthrene, were compared with those of sixteen US EPA priority PAHs. Two exposure times (6 or 24 h) were chosen to compare effects of metabolism of the studied PAHs on their AhR-inducing capacity.

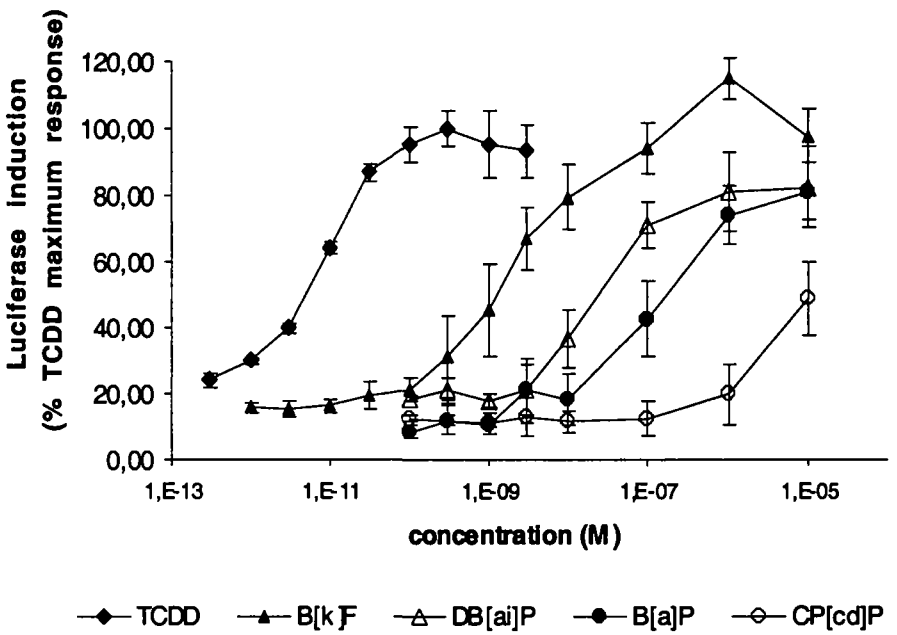

Figure 1: AhR-mediated induction of luciferase reporter gene in CALUX assay by TCDD, benzo[k]fluoranthene (B[k]F), dibenzo[a,i]pyrene (DB[ai]P), benzo[a]pyrene (B[a]P) and cyclopenta[c,d]pyrene (CP[cd]P) after 24 h exposure.

Due to the high rate of metabolism of PAHs, toxic equivalency factors or relative AhR-inducing potencies of these compounds are difficult to assess exactly. Therefore, the AhR induction potencies of PAHs were calculated as concentrations producing the same induction as the EC50 of reference inducers, i.e. TCDD and benzo[a]pyrene[6]. The derived induction equivalency factors allowed the following classification of the PAHs under study:

1. the strongest AhR inducers in the CALUX assay after 6 h exposure were indeno[c,d]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene and dibenzo[a,h]anthracene, in respective order;

2. the order of luciferase induction potency of the second, less potent group, was dibenzo[a,i]pyrene > dibenzo[a,e]pyrene > 5-methylchrysene > dibenzo[a,j]anthracene > dibenzo[a,h]pyrene; the last one showed only submaximum dose-response curve;
3. the third group of PAHs induced luciferase in the concentrations similar to benzo[a]pyrene in the following order: benzo[a]pyrene > 7,12-dimethylbenzanthracene > benz[a]anthracene > chrysene > dibenzo[a,l]pyrene;
4. a lower, but still significant, AhR-induction was found after the treatment with benzo[c]phenNTHRENE > cyclopenta[c,d]pyrene > benzo[g,h,i]perylene.

When using 24 h exposure, induction equivalency factors of the tested PAHs (relative to TCDD), were significantly lower in comparison to 6 h exposure. However, a similar order of luciferase induction potencies was observed with both exposure times, with exception of dibenzo[a,e]pyrene and dibenzo[a,l]pyrene whose induction potencies determined after 24 h exposure were much lower in comparison with their potencies after 6 h exposure. Figure 1 shows dose-response curves of reference compounds as well as those of representantive PAHs of each of the groups discussed above that were obtained in the CALUX assay.

Based on relative AhR-inducing potencies and data on concentrations of mutagenic PAHs in the environmental samples (data not shown), it can be concluded that indeno[1,2,3-c,d]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene, and dibenzo[a,h]anthracene form the most important group of dioxin-like active PAHs and are followed by 5-methylchrysene, dibenzo[a,i]pyrene, and dibenzo[a,e]pyrene that showed toxic equivalents similar to benzo[a]pyrene and chrysene. Dibenzo[a,l]pyrene and cyclopenta[c,d]pyrene, identified as the strongest in vitro mutagens[1], showed only weak AhR-inducing potencies.

Acknowledgement
This study was supported by the Czech Ministry of Environment (grant No. VaV/340/2/00).

References

1. Durant J.L., Busby W.F., Lafleur A.L., Penman B.W. and Crespi C.L. (1996) Mutation Res. 371, 123.
2. Durant J.L., Lafleur A.L., Busby W.F., Donhoffer L.L., Penman B.W. and Crespi C.L. (1999) Mutation Res. 446, 1.
3. Piskorska-Pliszczynska J., Keys B., Safe S. and Newman M.S. (1986) Toxicol. Lett. 34, 67.
4. Machala M., Mátlová L., Svoboda I. and Nezveda K. (1996) Arch. Toxicol. 70, 362.
5. Willett K.L., Gardinali P.R., Sericano J.L., Wade T.L. and Safe S.H. (1997) Arch. Environ. Contam. Toxicol. 32, 442.
6. Jones J.M., Anderson, J.W. (1999) Environ. Toxicol. Pharmacol. 7, 19.
7. Murk A.J., Leonards P.E.G., Bulder A.S., Jonas A.S., Rozemeijer M.J.C., Denison M.S., Koeman J.H. and Brouwer A. (1997) Environ. Toxicol. Chem. 16, 1583.