VI Seminario Nazionale per Dottorandi in Farmacologia e Scienze Affini
Siena, Certosa di Pontignano, 23 - 26 Settembre 2002

IN VITRO NEUROTOXICITY OF THE FLAME RETARDANT 2,2', 4,4',5-PENTABROMODIPHENYL ETHER (PBDE 99)

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The polybrominated diphenylethers (PBDEs) are a class of chemicals widely used as flame retardants in plastics and in textile coatings (Darnerud et al. 2001). PBDEs bioaccumulate in the environment, and PBDE residues have been found in sediments, marine mammals, fish, bird eggs, as well as human milk, serum, and adipose tissue (McDonald, 2002). The PBDEs commercial products consist of penta-,octa- and decabromodiphenyl ether mixtures. The fully brominated deca –BDE congener is poorly absorbed, rapidly eliminated and does not bioaccumulate. In contrast, the lower molecular weight congeners, tri- to hexa-BDEs, are almost completely absorbed, slowly eliminated, highly bioaccumulative and much more bioactive that deca BDE. Exposure to PBDEs has been associated with thyroid hormone disturbance (McDonald, 2002). Furthemore, there are preliminary evidences that PBDEs may be developmental neurotoxicants (Eriksson et al. 2001).
PBDEs are structurally related to polychlorinated biphenyls (PCBs), which cause developmental neurotoxicity in animals and humans (Tilson and Kodavanti, 1998). Though the exact mechanism of PCBs developmental neurotoxicity has not been established , there is evidence that *in vitro* these compounds may interfere with signal transduction systems in cultured cells (Kodavanti and Tilson, 2000).
Aim of this study was to conduct a preliminary investigation of the *in vitro* effect of PBDE 99 (a pentabromodiphenyl ether) on cultured cells. The human 132-1N1 human astrocytoma cells and rat cerebellar granule cells (CGC) were used for this purpose. Aroclor 1254 (a PCB) was used as positive control.
PBDE 99 (2,2', 4,4', 5- pentabromodiphenyl ether) caused a concentration-dependent inhibition of MTT in proliferating and quiescent astrocytoma cells. The IC_{50} values were 57.0 mM 57for Aroclor 1254 and 73.1 mM for PBDE 99 in proliferating cells, and 33.6 mM for Aroclor 1254 and 42.4 mM for PBDE 99 in quiescent cells. Measurements of LDH release (an indicator of membrane integrity) and Trypan blue dye exclusion assays, however, indicated that only the highest concentration of Aroclor 1254 (100 mM) had any effect in proliferating cells, while both 50 and 100 mM were cytotoxic in quiescent cells. PBDE 99 had no effect both in either quiescent or proliferating cells. Cytotoxicity was also determined in CGC by the MTT assay; IC_{50} values were 52.7 mM and 59.4 mM for Aroclor 1254 and PBDE 99, respectively.np2057
As PCBs have been found to affect protein kinase C and calcium homeostasis in CNS cells, we investigated whether Aroclor 1254 and PBDE 99 would alter the activity of PKC in human 132-1N1 astrocytoma cells. These cells express three isozymes of PKC, the classical PKC a, the novel PKC ee1040 and the atypical PKC z (Post et al. 1996). PBDE 99 (100 mM) causes translocation of all three isozymes from the cytosol to the membrane, while Aroclor 1254 (50 mM) 57causes translocation of only PKC a and PKC e.
GF 109203X (a competitive inhibitor for the ATP-binding site of PKC) did not modify PBDE 99- or Aroclor 1254-induced cytotoxicity in astrocytoma cells. Similarly, down regulation of a
langnp2057 and e PKCs by prolonged treatment with a phorbol ester, caused only a slight increase in the IC_{50} values for cytotoxicity. BAPTA AM (a cell permeable calcium chelator) had no effect on Aroclor cytotoxicity, but provide a slightfe1040 protection in case of PBDE 99.
These preliminary studies indicate that PBDE 99 and Aroclor 1254 are cytotoxic to glial and neuronal cells and suggest that PKCs and calcium may be only marginally involved.

References

1. Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. *Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology.* Environ Health Perspect. 2001 Mar;109 Suppl 1:49-68.

2. Eriksson P, Jakobsson E, Fredriksson A. *Brominated flame retardants: a novel class of developmental neurotoxicants in our environment?* Environ Health Perspect. 2001 Sep;109(9):903-8.

3. McDonald Thomas A. *A perspective on potential health risks of PBDEs.* Chemosphere 2002;46 745-755.

4. Tilson HA, Kodavanti PR.*The neurotoxicity of polychlorinated biphenyls*. Neurotoxicology. 1998 Aug-Oct;19(4-5):517-25.

5. Kodavanti PR, Tilson HA. *Neurochemical effects of environmental chemicals: in vitro and in vivo correlations on second messenger pathways.* Ann N Y Acad Sci. 2000;919:97-105

6. Post GR, Collins LR, Kennedy ED, Moskowitz SA, Aragay AM, Goldstein D, Brown JH. *Coupling of the thrombin receptor to G12 may account for selective effects of thrombin on gene expression and DNA synthesis in 1321N1 astrocytoma cells.* Mol Biol Cell. 1996 Nov;7(11):1679-90.
