Letter to the Editor

Assessing Adverse Effects of Aroclor 1254 on Perinatally Exposed Rat Offspring*

TANG Wei, CHENG Jin Ping#, YANG Yi Chen, and WANG Wen Hua

To assess the neurotoxic effects and redox responses of Aroclor 1254 (A1254) on perinatally exposed rat offspring, A1254 was administered by gavage from gestational day (GD) 6 to postnatal day (PND) 21. Neurobehavioral development, antioxidant enzyme activities, lipid peroxidation (LPO), nitric oxide (NO), and NO synthase (NOS) levels were analyzed in the offspring. Neurobehavioral development analysis revealed delayed appearance of the righting reflex, negative geotaxis, and cliff drop test responses in A1254 exposed group. Developmental A1254 exposure also caused oxidative stress in the brain of PND 22 offspring *via* reductions in the activity of SOD and GSH-Px, and by promoting a rise in the levels of NO and NOS.

Because they are persistent, widely distributed environmental contaminants, the potential toxic effects of polychlorinated biphenyls (PCBs) have been investigated from numerous perspectives (e.g., spatial memory deficits and endocrine-disrupting effects)[1]. Several studies have indicated that neonates may be exposed to PCBs through maternal milk[2]. Previous behavioral experiments on laboratory animals treated with PCBs have suggested that PCB-induced toxicity may be caused by interference with neurotransmission[3]. Neurotoxic effects observed in rats exposed to pollutants might depend on interactions among nitric oxide (NO), NO synthases (NOS), super oxide dismutase (SOD), and glutathione peroxidase (GSH-Px)[4]. However, few studies have fully explored the mechanisms related to the neurotransmission levels and antioxidant enzyme activities. Therefore, it is very important to identify the oxidative stress and neurotransmission pathways involved in exposure to PCBs, which are potential risk factors for neurotoxicity.

In order to assess the short- and medium-term adverse effects of perinatal exposure of rat offspring to A1254 (a commercial mixture of PCBs), the levels of SOD, GSH-Px, LPO, NO, and NOS in the brain, liver, and kidney were determined. In addition, we carried out righting reflex, cliff drop, and negative geotaxis tests to study the effects on neurobehavioral development.

The dose of A1254 (1.5 mg/kg) in this study was approximately 10 times higher than the mean level of ΣPCBs in female human adipose tissue. The period of administration [gestational day (GD) 6 to postnatal day (PND) 21] was chosen because it encompasses the formation of the first central nervous system areas, during which time indirect exposure to compounds through the mother ends. Only female mice were used in this study considering the fact that exposure to PCBs reduced the level of sexual receptivity in female offspring, but had no detectable effects on the sexual behavior of male offspring[5]. We chose the two anti-oxidant enzymes (SOD and GSH-Px) in our study because these two major antioxidants are involved in protection against oxidative stress and lipid peroxidation (LPO). NOS plays an important role in neurotransmission in the central and peripheral nervous systems. After being synthesized by NOS, in the body only, NO acts as a physiological messenger (like a neurotransmitter). Both of them were also detected in the present study using biochemical methods.

Ten primiparous Institute of Cancer Research mice obtained from Shanghai Animal Experimental Centre of China Science Institute were selected on GD 1. They were housed individually in a room in a 12-h light/12-h dark cycle maintained at 20 °C with free access to food and water. On GD 5, female mice were randomly divided into control (n=5) and exposure groups (n=5). A1254 (Accustandard, Lot #124-191) dissolved in corn oil (purity 96%; Shanghai), at a concentration of 1.5 mg/mL, was administered daily to the exposure group by gavage

doi: 10.3967/bes2015.097

*This work was supported by the National Natural Science Foundation of China (No. 21177087).

School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

[1.5 mg/(kg·d)] from GD 6 to PND 21, except for PND 0, at which time the dams were left undisturbed. Control animals were dosed with the vehicle only, during the same period. Within 24 h of birth, a litter was randomly reduced to six female neonates, which were then maintained by a dam. On PND 21, the pups were weaned and littermates were segregated and housed in plastic cages. Every two days, from PND 3-21, all pups from each litter of each treatment group, were used for postnatal assessment of neurobehavioral development. On PND 22, three female pups were randomly selected from six female pups of each treatment group, and euthanized for biochemical analysis. The remaining pups were sacrificed on PND 35. Methodological details of sample treatment, neurobehavioral analysis and biochemical analysis, including SOD, GSH-Px, NO, and NOS, were provided in our previous studies[4]. Data were subjected to analysis of variance (ANOVA). The differences were regarded as significant at $P<0.05$.

The analysis of reflexes and responses revealed that perinatal A1254 exposure strongly impaired neurobehavioral development of the offspring. Significantly delayed appearance was observed for the righting reflex, negative geotaxis, and cliff drop test responses in the exposed group (χ^2-test, $P<0.01$, Figure 1). Changes in these responses could reflect dysfunction in the peripheral nerves, spinal cord, cerebellum, thalamus, or cortical regions[6].

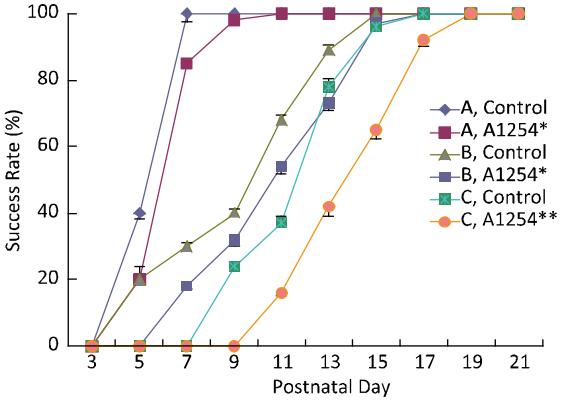

Figure 1. Neurobehavioral development of pups after perinatal exposure to A1254 or vehicle: (A) Righting reflex, (B) Cliff drop test, (C) Negative geotaxis; Bars represent mean±SEM, n=30 pups/group; Significant differences between the A1254 group and a control group on PND 22 at $^{*}P<0.05$, $^{**}P<0.01$.

Perinatal exposure to A1254 significantly decreased GSH-Px and SOD activities of offspring on PND 22 relative to controls ($P<0.05$) in the cerebral cortex, while the liver and kidney showed significant increases in GSH-Px and SOD levels in offspring treated with A1254 ($P<0.05$, Table 1).

Oxidative stress developed when there was an imbalance between the ratio of pro-oxidants and anti-oxidants, which led to the accumulation of oxidative damaged molecules. The oxidative stress-induced damage depended on the balance between the magnitude of the stress and the effectiveness of the antioxidant enzymes. A significant rise in the level of SOD activity in the liver and kidney seems to combat the excessive generation of superoxide anions, which are further neutralized by GSH-Px enzymes[7]. The cerebral cortex is rich in non-heme iron, which is catalytically involved in the production of oxygen-free radicals, thus increasing the risk of neurodegenerative diseases[8]. Therefore, decreased levels of antioxidant enzyme activity in the PCB-exposed group on PND 22 indicated an increase in oxidative stress in the cerebral cortex. This indicates an adaptive response of the redox-defense system in the liver and kidney, as opposed to a general breakdown of the redox defense system in the brain, after A1254 exposure.

Age-related differential levels of GSH-Px and SOD showed that GSH-Px and SOD activities in the cerebral cortex, liver, and kidney were significantly increased in PND 35 pups compared to their levels in PND 22 pups (Table 1, $P<0.05$). The variation in the cerebral cortex, liver, and kidney SOD and GSH-Px activities with age suggests that SOD and GSH-Px production may be regulated during the development of these organs. PCBs accumulate in adipose tissue and are transferred via the mother's milk to her offspring during neonatal development[2]. The transfer of environmental contaminants *via* human milk is one of the major routes of exposure to young humans. Thus, after weaning for 14 d (on PND 35), these changes in the activity of antioxidant enzymes were restored to control group levels.

LPO is one of the main manifestations of oxidative damage and plays an important role in the toxicity of many xenobiotics in vertebrates[8]. Increased levels of LPO generation were observed in different tissues (cerebral cortex, liver, and kidney) in the A1254 treated group of PND 22 rats (Table 2). However, the level of LPO generation in the liver and kidney did not differ significantly between the

exposed group and controls. NO is synthesized by NOS in the body only, acts as a physiological messenger (like a neurotransmitter), and as a neurotoxic agent in the central nervous system. NO levels and NOS activity in the cerebral cortex, liver, and kidney in the treatment groups w significantly increased relative to those in the control groups (P<0.05; Table 2) on PND 22 and PND 35. Age-related differential content of NO in these tissues showed that NO levels were significantly less in PND 35 pups than in PND 22 pups (P<0.01). However, compared to PND 22 pups, NOS in these tissues were significantly greater in PND 35 pups (P<0.05).

Changes in antioxidant enzyme activities and neurotransmitter levels have been proposed to affect neurobehavior[9]. In this study, one of the mechanisms of the developmental neurotoxic effects of A1254 can involve changes in neurotransmitter levels and antioxidant enzyme activities.

SOD protects against damage due to oxygen-free radicals by catalyzing the removal of the superoxide radical ($O_2\cdot$), which damages membranes and biological structures. The decrease in SOD activity in the cerebral cortex might have resulted in greater accumulation of $O_2\cdot$. However, excess NO can react rapidly with $O_2\cdot$ to form a potent long-lived oxidant, peroxynitrite ($ONOO\cdot$). This can interact with nucleic acids, proteins, and lipids, and contribute substantially to the cellular redox state[10]. GSH-Px metabolizes peroxides, such as H_2O_2, and protects cell membranes from LPO[8]. In the present study, the

Table 1. The Activities of GSH-Px and SOD in Cerebral Cortex, Liver and Kidney of Rat Offspring on PND 22 and PND 35 following Exposure to A1254 or Vehicle from GD 6 to PND 21

Postnatal Day		PND 22		PND 35	
		Control	A1254	Control	A1254
Cerebral cortex	GSH-Px (U/mg protein)	18.2±1.1	12.1±0.6**	23.1±1.9$^{\#}$	22.4±1.2$^{\#\#}$
	SOD (U/mg protein)	13.4±0.5	9.6±0.9**	24.6±0.4$^{\#\#}$	23.5±0.4$^{\#\#}$
Liver	GSH-Px (U/mg protein)	14.4±0.7	18.0±1.8*	20.9±1.2$^{\#\#}$	22.1±1.3$^{\#}$
	SOD (U/mg protein)	15.3±0.8	20.5±1.2*	34.2±0.9$^{\#\#}$	31.9±1.3$^{\#\#}$
Kidney	GSH-Px (U/mg protein)	11.1±0.6	13.9±0.8*	19.3±0.5$^{\#\#}$	21.9±1.2$^{\#\#}$
	SOD (U/mg protein)	12.7±0.4	17.3±0.7*	19.4±0.8$^{\#}$	21.6±1.0$^{\#\#}$

Note. Data are presented as mean±SEM. n=15 pups/group. Significant differences between A1254 group and control group on PND 22 at $^{*}P$<0.05, $^{**}P$<0.01. Significant differences between PND 22 group and PND 35 group at $^{\#}P$<0.05, $^{\#\#}P$<0.01.

Table 2. Levels of LPO, NO, and NOS in Cerebral Cortex, Liver and Kidney of Rat Offspring on PND 22 and PND 35 following Exposure to A1254 or Vehicle from GD 6 to PND 21

Postnatal Day		PND 22		PND 35	
		Control	A1254	Control	A1254
Cerebral cortex	LPO (nmol/mg protein)	1.91±0.42	3.08±0.23**		
	NO (μmol/L)	17.52±2.52	24.48±3.52**	11.45±0.77$^{\#\#}$	15.60±0.64$^{\#\#}$
	NOS (U/mL)	9.10±1.22	13.46±1.56*	15.47±1.49$^{\#\#}$	18.51±2.97$^{\#\#}$
Liver	LPO (nmol/mg protein)	1.97±0.41	2.24±0.25		
	NO (μmol/L)	21.12±2.69	26.53±3.24*	12.54±1.20$^{\#\#}$	17.46±0.56$^{\#\#}$
	NOS (U/mL)	11.24±1.06	20.05±4.14**	21.04±4.02$^{\#\#}$	29.28±4.26$^{\#\#}$
Kidney	LPO (nmol/mg protein)	2.40±0.12	2.47±0.21		
	NO (μmol/L)	28.97±5.08	32.46±3.58*	15.05±0.95$^{\#\#}$	19.51±0.27$^{\#\#}$
	NOS (U/mL)	9.76±0.97	16.53±0.43**	13.22±1.39$^{\#}$	20.11±1.94$^{\#\#}$

Note. Data are presented as mean±SEM. n=15 pups/group. Significant differences between A1254 group and control group on PND 22 at $^{*}P$<0.05, $^{**}P$<0.01. Significant differences between PND 22 group and PND 35 group at $^{\#}P$<0.05, $^{\#\#}P$<0.01.

observed decline in the activity of GSH-Px in the cerebral cortex of A1254 exposed animals may be ascribed to an increase in the level of LPO (Table 2). The increased LPO observed in the cerebral cortex in this study, may also have been caused by decreased SOD activity. The decrease in SOD activity in the cerebral cortex might have resulted in greater accumulation of free radicals. It could be an auto-destructive mechanism in the cerebral cortex, which has a very important role in mediating balance and coordination. This might have upset the pro-oxidant/anti-oxidant balance within the cerebral cortex, which could be one of the main reasons for the increase in oxidative stress in the cerebral cortex of the exposed group.

The results of this study suggest that developmental A1254 exposure causes serious impairment of neurological development in developing rats. Significantly, delayed appearance of the righting reflex, negative geotaxis, and cliff drop test responses could reflect dysfunction in the cortical regions. The present work demonstrated that developmental A1254 exposure caused oxidative stress, which developed when there was an imbalance between pro-oxidants and anti-oxidants ratio in the cerebral cortex of PND 22 offspring by decreasing SOD and GSH-Px activity, and increasing the levels of LPO, NO, and NOS. However, these changes were not permanent, and antioxidant enzyme activities returned to control levels on PND 35.

[#]Correspondence should be addressed to CHENG Jin Ping, PhD, Tel: 86-21-54742823, E-mail: twsywt@163.com; jpcheng@sjtu.edu.cn

Biographical note of the first author: TANG Wei, male, born in 1987, Master Degree, majoring in ecotoxicology and environmental chemistry.

Received: February 2, 2015;

Accepted: September 10, 2015

REFERENCES

1. Meeker JD, Hauser R. Exposure to polychlorinated biphenyls (PCBs) and male reproduction. Syst Biol Reprod Med, 2010; 56, 122-31.
2. Park JS, Linderholm L, Charles MJ, et al. Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in pregnant women from eastern Slovakia. Environ Health Persp, 2007; 115, 20-7.
3. Selvakumar K, Bavithra S, Ganesh L, et al. Polychlorinated biphenyls induced oxidative stress mediated neurodegeneration in hippocampus and behavioral changes of adult rats: Anxiolytic-like effects of quercetin. Toxicol Lett, 2013; 222, 45-54.
4. Cheng JP, Gu JM, Ma J, et al. Neurobehavioural effects, redox responses and tissue distribution in rat offspring developmental exposure to BDE-99. Chemosphere, 2009; 75, 963-8.
5. Wang XQ, Fang J, Nunez AA, et al. Developmental exposure to polychlorinated biphenyls affects sexual behavior of rats. Physiol Behav, 2002; 75, 689-96.
6. Kuriyama SN, Talsness CE, Grote K, et al. Developmental exposure to low-dose PBDE-99: effects on male fertility and neurobehavior in rat offspring. Environ Health Persp, 2005; 113, 149-54.
7. Karthikeyan S, Sridhar M, Ramajayam G, et al. Polychlorinated biphenyl (PCBs)-induced oxidative stress plays a role on vertebral antioxidant system: Ameliorative role of vitamin C and E in male Wistar rats.Biomed Prev Nutr, 2014; 4, 411-6.
8. Venkataraman P, Muthuvel R, Krishnamoorthy G, et al. PCB (Aroclor 1254) enhances oxidative damage in rat brain regions: protective role of ascorbic acid. Neurotoxicology, 2007; 28, 490-8.
9. Viberg H, Fredriksson A, Eriksson P. Neonatal exposure to the brominated flame-retardant, 2,2',4,4',5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. Environ Toxicol Phar, 2004; 17, 61-5.
10. Radi R, Peluff G, Alvarez MN, et al. Unraveling peroxynitrite formation in biological systems. Free Radical Bio Med, 2001; 30, 463-88.