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No evidence for oxidative stress in the cerebellar tissues or cells of juvenile male mice exposed via lactation to the 6 non-dioxin-like PCBs at levels below the regulatory safe limits for humans

Arpiné Ardzivian Elnar[a,c,*], Frédéric Desor[a,c], Sylvain Legay[d], Christophe Nemos[e], Frances T. Yen[b,c], Thierry Oster[b,c], Torsten Bohn[d], Rachid Soulimani[a,c]

[a] Neurotoxicologie Alimentaire et Bioactivité, MRCA, BP 4102, 57040 Metz, France
[b] Biodisponibilité et Fonctionnalités des Lipides Alimentaires, BFLA, ENSAIA, Avenue de la forêt de Haye, 54500 Vandoeuvre-lès-Nancy, France
[c] Université de Lorraine, Unité de Recherche Animal et Fonctionnalités des Produits Animaux (UR AFPA), EA3998, INRA USC 0340, France
[d] Luxembourgish Institute of Science and Technology, Environmental Research and Innovation Department, 41, rue du Brill, L-4422 Belvaux, Luxembourg
[e] Université de Lorraine, UMR INSERM-954 Nutrition - Génétique et exposition aux risques environnementaux, Faculté de médecine de Nancy, 54511 Vandœuvre-Lès-Nancy, France

HIGHLIGHTS

- We exposed dam mice to ∑6 NDL-PCBs (0, 1, 10 and 100 ng/kg b.w./day; PNDs 0–14).
- Oxidative status was evaluated in the cerebellum of PCB lactationally exposed pups at PND 14.
- No differences in ROS production, oxidative stress-related gene expressions or protein levels were shown between groups.
- Lactational exposure to ∑6 NDL-PCBs did not induce an oxidative damage in male mice at brain growth spurt.

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ABSTRACT

The developing central nervous system is particularly vulnerable to environmental contaminants such as non-dioxin-like polychlorinated biphenyls (NDL-PCBs). This study investigated the potential oxidative effects in mice pups exposed via lactation to the sum of the six indicator NDL-PCBs (∑6 NDL-PCBs) at 0, 1, 10 and 100 ng/kg per 14 days, constituting levels below the guidance values fixed by French food safety agencies for humans at 10 ng/kg body weight per day. For this purpose, the oxidative status was assessed by flow cytometry via dichloro-dihydro-fluorescein diacetate in the cerebellum of juvenile male offspring mice during brain growth spurt [postnatal day (PND) 14]. No significant differences were found in the levels of reactive oxygen species in the cerebellar neurons or glial cells (astrocytes, oligodendrocytes and microglia) of lactationally exposed male mice at PND 14 ($p > 0.05$). Concordantly, oxidative-stress related gene expression was measured by qPCR for catalase, copper zinc superoxide dismutase 1, glyoxalase 1, glutathione peroxidase 1, and glutathione reductase 1, in the cerebellum at PND 14 appeared unaffected, as also verified at the protein level by immunoblots. Moreover, transcriptomic data from our previous work have not shown differences in the mRNA expressions of genes belonging to GO terms involved in oxidative stress in neurons of male mice exposed to ∑6 NDL-PCBs compared to controls; except for glyoxalase 1 which was downregulated in neurons isolated from exposed group compared to controls. Our findings suggest that lactational exposure to NDL-PCBs at environmental relevant concentrations may not cause significant oxidative effect on juvenile cerebellum.

1. Introduction

Non-dioxin-like polychlorinated biphenyls (NDL-PCBs) are persistent organic chemicals that accumulate in the environment and biological tissues. Once stored in adipose tissues, they are

* Corresponding author at: Université de Lorraine, BP 4102, 57040 Metz, France.
E-mail addresses: arpine.elnar@hotmail.fr, arpine.el-nar@univ-lorraine.fr (A.A. Elnar).

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transferred from the mother to the offspring via breastfeeding (Vigh et al., 2013). This transfer may contribute to 5–10% of the total body burden of NDL-PCBs observed at adult age (Afssa-Avis, 2015), and results in critical vulnerability during neonatal life, altering maturation processes in the developing central nervous system (Grandjean and Landrigan, 2014). In humans, rapid development in the brain spans from the third trimester of gestation and ends by the third year of life while in mice, it occurs within the first month of life, peaking at 10 days of age (Dobbing, 1975). During this period often called the "brain growth spurt"., the developing brain is more vulnerable to toxic insults than the adult brain. NDL-PCBs can alter neuron functioning, resulting in long-lasting changes to the brain (Elnar et al., 2015b; Kodavanti, 2005; Pessah et al., 2010).

We have recently reported persistent anxiety-like behavior in male mice lactationally exposed to environmentally plausible levels (1, 10 and 100 ng/kg/day) of the sum of the six indicator NDL-PCBs (PCBs 28, 52, 101, 138, 153 and 180; Elnar et al., 2012). These 6 NDL-PCBs, representing the most predominant PCB congeners in contaminated fish matrices (EFSA, 2012, 2010), have also shown to increase the expression of ryanodine receptor 3 (RyR3) in the cerebellum of the juvenile mice (Elnar et al., 2012), with a p53-dependent response to cellular stress and a decrease in the expression of proteins involved in the transmission of electrical signals in neurons of the exposed mice (Elnar et al., 2015a).

In general, it has been shown that the developing brain has lower levels of antioxidants (Tian et al., 1998) and that the antioxidant system in young animals is less efficient than in adults. Thus, a decrease in antioxidant enzyme activity could be expected following the exposure to environmental insults, such as NDL-PCBs (Vicente et al., 2004). In addition, many studies have shown the well-known pro-oxidant effect of PCB commercial mixtures such as Aroclor 1254 (Venkataraman et al., 2010), whose constitution is relatively high in NDL-PCB congeners (Frame et al., 1996a,b). Given the link between oxidative stress and anxiety (Bouayed et al., 2009), we hypothesized that exposure to NDL-PCBs could also have a putative mechanism by which it may disrupt the oxidative status of neurons, at a period where the central nervous system is particularly susceptible to free radical related oxidative damage (Buonocore et al., 2001). This could be responsible for the anxiety-like behavior observed at more advanced life stages.

Thus, in the present investigation, we examined a new set of F1 male mice whose mothers were lactationally exposed to $\sum$6 NDL-PCBs and their controls, in order to evaluate the level of intracellular oxygen-derived species in neurons and glial cells. In addition, gene expression and protein changes of stress-related enzymes, important in the detoxification of oxygen radicals such as catalase (Cat), copper-zinc superoxide dismutase 1 (Cu/ZnSod1), glyoxalase 1 (Glo1), glutathione peroxidase 1 (GPx1), and glutathione reductase 1 (Gsr1), were investigated in the cerebellar tissues of the juvenile male mice at brain growth spurt, i.e. at postnatal day (PND) 14, the end of the exclusive lactational exposure.

2. Materials and methods

2.1. Chemicals

The fluorescent probe dichloro-dihydro-fluorescein diacetate (DCFH-DA) was purchased from Sigma–Aldrich (Saint Louis, MO, USA). The congeners 2,4,4′-trichlorobiphenyl (PCB 28; batch no. SZE6317X; 99.9% purity), 2,2′,5,5′-tetrachlorobiphenyl (PCB 52; batch no. SZE6234X; 99.6% purity), 2,2′,4,5,5′-pentachlorobiphenyl (PCB 101; batch no. SZE6298X; 99.9% purity), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB 138; batch no. SZE7089X; 99.6% purity), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153; batch no. SZE9271X; 99.5% purity) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180; batch no. SZE9105X; 99.5% purity) were obtained from Sigma–Aldrich Co. (St. Quentin Fallavier, France, purity >99%). Hank's balanced salts solution (HBSS) was purchased from Sigma–Aldrich. MACS buffer and neural tissue dissociation kit (papain and trypsin) were acquired from Miltenyi Biotec (Bergisch Gladbach, Germany).

2.2. Animals and treatment

In this study, we used twenty female and twenty male Swiss Albino mice (OF1, Janvier; France), aged nine weeks, and weighing between 30 and 40 g. Animals were housed with five mice/cage/sex under a 12-h light/12-h dark schedule (lights on at 8:00 pm), with free access to water and food (SDS Dietex; France). Mice were maintained at a constant temperature (22 ± 2°C) and a relative humidity of 55 ± 10%. Following an acclimatization period of one week, female mice were mated overnight and examined the following morning for copulatory plugs. The fertilization rate was 80% (16 of 20). Gravid females were individually housed and were assigned to experimental groups by stratified randomization ($n = 4$ dams/group). A cotton nest square as a source of nesting material was supplied one week before the delivery. The day of parturition was considered as PND 0. At this date, litters were culled to ten pups with equal sex ratio, when possible. The dams' weight was recorded daily to adjust treatment levels.

Mice dams received 0, 1, 10 and 100 ng/kg b.w./day of a mixture of pure molecules of $\sum$6 NDL-PCBs [PCBs 28 (2%), 52 (6%), 101 (12%), 138 (32%), 153 (37%), and 180 (11%)] in rapeseed oil (10 ml/kg b.w.) by oral gavage, starting from parturition (PND 0) until the end of exclusive lactation (PND 14). The preparation of the stock solution of $\sum$6 NDL-PCBs and the composition of the 6 NDL congeners (PCBs 28, 52, 101, 138, 153 and 180) in the mixture were detailed previously (Elnar et al., 2012).

On PND 14, only two males of the 10 pups were randomly selected from each litter for analyses, anaesthetized with halothane, and then sacrificed, taken into account the litter as unit of variance in this type of study and to avoid the litter effect (Holson et al., 2008; OECD, 2007).

One male pup per litter was used for cytometric analysis of isolated cerebellar cells ($n = 4$/group), and the second male pup from the same litter was used for detection of mRNA and protein expression of enzymes from the whole cerebellar tissues ($n = 4$/group). Cerebellar tissues were homogenized in RLT buffer [RNeasy mini kit (Qiagen; Leusden, The Netherlands)] by vortex using two sterile metallic balls per sample (that had been cooled with liquid nitrogen). Tissues were snapped frozen in liquid nitrogen, and then stored at −80°C until RNA and protein extraction and analysis. All animal use and care procedures were performed in accordance with the Directive 2010/63/EU, and were approved by the local research ethics committee of the University of Lorraine (CELMEA-2013–0010).

2.3. Assessment of oxidative status in cerebellar neurons and glial cells

2.3.1. Neuron and glial cell dissociation

On PND 14, the cerebellum of male mice was excised from the brain in order to isolate neurons or glial cells, according to the manufacturer's protocol (Miltenyi Biotec). In brief, the cerebellar tissue was removed, homogenized using a potter in 1 ml of cold HBSS. The cell suspensions were centrifuged ($300 \times g$, 4°C, 2 min), and the supernatants were discarded. The tissues were dissociated by incubation with papain (for neuron and oligodendrocyte collection) or with trypsin (for astrocyte and microglia isolation) as described in the neural tissue dissociation kit. Cells were manually triturated, and were passed through a 30 μm nylon mesh (pre-separation filters, Miltenyi Biotec) to remove cell clumps. After centrifugation of the solutions ($300 \times g$, 4°C, 10 min), the supernatants were