PERINATAL EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS: PARALLELS IN SEALS AND HUMANS

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Introduction

The young of many species are vulnerable to the immune- and endocrine-disrupting effects of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dioxins and furans (PCDD/Fs), and organohalogenated pesticides through placental and lactational exposure[1]. In numerous animal species, these lipophilic chemicals can cross the placenta, accumulate in fetal and neonatal tissues, and exert subtle but persistent effects on the developing immune system, reproductive system, thyroid, and brain [2-5]. In human infants, alterations in T cells, thyroid hormones, and neurological and cognitive development have been reported at concentrations found in cord blood and breast milk of fish-consumers [6-10]. Children of native Inuit women from Arctic Quebec who consume fatty fish and contaminated marine mammal products may be at higher risk. Dewailly and colleagues[11] reported that pre- and postnatal exposure to PCBs and pesticides is associated with a significantly increased incidence of middle ear infections (*otitis media*) in Inuit infants that can persist into childhood.

Although interspecies comparisons can be complicated by differences in metabolism, duration of lactation, feeding habits, and general health status, congener-specific analyses of PCBs and PCDD/Fs in marine mammal tissues indicate that the uptake and toxicokinetic disposition of these compounds is qualitatively similar for humans and seals[12,13]. Neurodevelopmental endpoints have not been investigated in seals; however, captive feeding studies have demonstrated that moderate burdens of PCBs and PCDD/Fs can adversely affect normal immune function and thyroid hormone homeostasis in adult harbor seals[14-16]. These studies supported the hypothesis that widespread PCB-induced immunosuppression in European seals played a major role in the large-scale morbillivirus outbreaks of the late 1980s. Results of a recent field study of neonatal harbor seals[17] indicate that the threshold levels for subtle effects of dioxin-like PCBs on immune and endocrine parameters may be quite low in young seals, similar to the levels at which subtle effects have been reported in human infants. The parallels observed in neonates from these two species suggest that harbor seals may be a sensitive and appropriate wildlife model for understanding the adverse effects of perinatal exposure to dioxin-like compounds in the developing child.

Methods and Materials

Peripheral blood and blubber samples were obtained from 11 harbor seal pups that had stranded as newborns along the central California coast and were housed at a rehabilitation facility. All pups were exposed primarily *in utero*. Whole blood and serum samples were collected for immune function and endocrine studies when pups were ~4 weeks old. Health status was evaluated at the same time to control for disease. Blubber biopsies (1g) were taken from the dorsal region using a 6 mm dermal punch. Larger samples (10g) were collected from the mid-ventral region at necropsy, and

frozen at -40°C until analysis. PCB congeners including coplanar PCBs and chlorinated pesticide residues were analyzed in blubber by GC/ECD using a porous graphitic carbon (PGC) column methodology[18]. Immune function was evaluated using a whole blood assay to measure *in vitro* lymphocyte proliferative responses to Conconavalin A (Con A), phytohemagglutinin (PHA) and poke weed mitogen (PWM)[19]. Standard radioimmunossays (RIA) using commercially available kits (Amerlex-MAB, Amersham, England) were used to measure total and free thyroxine (T^4, FT^4) and free triiodothyronine (FT^3) concentrations in seal serum. Total triiodothyronine (T^3) was measured in seal serum using a standard assay previously validated in domestic animals[20]. Relationships between exposure and outcome measures were evaluated using chi square statistics, bivariate correlation statistics, ANOVA and MANOVA with Bonferroni criteria, and multiple regression analyses.

Results and Discussion

PCB levels in harbor seal pup blubber ranged from 1.4 – 5.3 ppm (mean 3.3 ppm, lipid weight basis), and DDE levels ranged from 0.7 – 13.4 ppm (mean 3.9 ppm). Hexachlorobenzene (HCB) was detected in blubber at trace levels. While PCB levels in the pups were relatively low, the coplanar PCBs 77, 81, and 126 contributed 46% of their PCB-TEQ burdens (range 25 – 262 ppt, mean 88 ppt), and were comparable to or higher than levels previously reported in adult harbor seals from heavily polluted areas and those of captive harbor seals with impaired immune responses following dietary exposure to contaminated fish[13]. Whether the coplanar PCB pattern in these pups reflects a high maternal transfer or a reduced metabolic capacity for these congeners in the neonate is unclear. Previous studies have reported lower MC-type P-450 monooxygenase activities in neonatal seals than in adults[21], suggesting that neonates may retain even the more metabolizable coplanar congeners during a period of development when they are most sensitive to their effects.

Using regression analysis, higher PCB-TEQ levels in blubber of the harbor seal pups were significantly correlated with reduced lymphocyte proliferative responses to all three mitogens (Con A: R^2= 0.42, p=0.04; PHA: R^2= 0.45, p=0.03; PWM: R^2= 0.46, p=0.03), suggesting an effect of dioxin-like PCBs on neonatal T cell function. These findings agree with the results of the feeding experiment in which lymphocyte proliferative responses to mitogens were negatively correlated with TEQ levels in blubber of captive harbor seals fed contaminated fish[14]. Immune responses to Con A and PWM in the pups were also negatively correlated with blubber levels of DDE (Con A: R^2= 0.47, p=0.04; PWM: R^2= 0.54, p=0.02). Similar negative correlations between *in vitro* immune responses and PCB and DDT/DDE levels in plasma were reported in a field study of free-ranging bottlenose dolphins [22].

Serum thyroid hormone levels in the pups were negatively correlated with blubber concentrations of PCB-TEQ (FT^4: R^2= 0.53, p=0.02) and sum PCBs (T^3: R^2= 0.74, p=0.01), suggesting a possible involvement of both planar and nonplanar PCB congeners. These findings are consistent with results of an earlier feeding study[16] in which thyroid hormones (T^4, FT^4, and T^3) were reduced in tandem with retinol levels in plasma of harbor seals with moderate contaminant burdens. The suggested mechanism for these alterations was via competitive interference of OH-PCBs with a common carrier protein. Serum retinol levels in the pups were also negatively correlated with PCB-TEQ levels in blubber (R^2= 0.46, p=0.04), which agrees with previous observations that retinol is a sensitive marker of organochlorine exposure in harbor seals[14,16]. It is also possible that the pups' limited maternal contact (and colostrum deprivation) may have affected vitamin A stores in the liver.

Comparisons in Seals and Humans

In Dutch infants, a higher prenatal PCB/dioxin exposure was associated with alterations in T cell subpopulations in the blood[6]. These changes consisted of increases in the number of activated T cells at birth and an increase in the total number of T cells and the number of $CD8^+$ (cytotoxic) T cells as well as activated T cells at 18 months of age. A higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts at 3 months of age. In Inuit infants, prenatal exposure to PCBs and chlorinated pesticides was significantly associated with the risk of otitis media during the first year of life[11]. Associations were most consistent with *p,p'*-DDE (mean ~ 1.2 ppm in breast milk fat) and HCB (mean 0.1 ppm), and over time, were more frequent among breast fed infants receiving the highest organochlorine dose than in bottle fed infants. These findings indicate that background pre- and postnatal exposure to organochlorines may influence the human fetal and neonatal immune system, possibly predisposing the newborn to opportunistic infections.

In the harbor seal pups, the PCB and PCB-TEQ levels associated with reduced *in vitro* immune responses were very similar to the levels associated with altered T cells in the Dutch infants. For the infants, contaminant levels were expressed indirectly as mean PCB 2.2 ppm in maternal plasma, and sum TEQ of 67 ppt in milk lipids for coplanar PCBs and 17 PCDD congeners, as compared with the levels in seal pup blubber (PCB 3.3 ppm and sum PCB-TEQ of 88 ppt lipid weight basis). In the Dutch study, the changes in T cells were not correlated with the incidence of infections or impaired antibody responses to pathogenic exposures in the infants. This is in contrast to the Inuit study in which a higher organochlorine (DDE, HCB) exposure was associated with the incidence of disease, but not with changes in immunologic parameters. In the harbor seal pups, higher PCB-TEQ and DDE levels in blubber were correlated with reduced *in vitro* immune responses (a marker of immune function), and contaminant levels were higher (although not significantly) in pups with infectious disease.

In a separate study of Dutch infants, maternal burdens of PCBs and dioxins (PCB ~ 2 ppm in maternal plasma) were associated with alterations of thyroid hormone homeostasis[7]. A higher prenatal exposure to dioxins and PCB-TEQ levels in breast milk was significantly correlated with lower maternal plasma T^4 and T^3 levels and with higher TSH levels in the infant's plasma at the age of 2 weeks and 3 months. In infants with a higher exposure, plasma T^4 levels were significantly lower (10%) and TSH levels were significantly higher (37%) at 2 weeks of age. Similarly, in the 4-week-old harbor seal pups, higher blubber levels of PCBs were significantly correlated with lower T^3 levels and higher PCB-TEQ levels were correlated with lower FT^4 levels in serum. These results suggest that prenatal exposure to PCBs and dioxins at current background levels may pose a risk for altered thyroid hormone homeostasis in the neonate.

Contaminant-related Responses in Neonatal Harbor Seals and Human Infants

Population	Exposure Route/Level (lipid basis)	Tissue Analyzed		Trend	Reference
Harbor seal pups	Prenatal PCB-TEQ ~88 ppt DDE ~ 4 ppm	Blubber	↓ ↑	T/B cell mitogen responses Incidence of infections	17
	PCB-TEQ ~88 ppt PCBs ~ 3 ppm	Blubber	↓ ↓	Thyroid hormones (FT^4, T^3) Retinol (Vitamin A)	17
Dutch infants	Perinatal PCBs/PCDD/Fs PCBs ~2 ppm TEQ ~67 ppt	Milk fat	↑ ↑ ↓	T helper cells T cytotoxic cells Monocytes, granuloctyes	6
	(for PCBs + PCDD/Fs)	Milk fat	↓ ↑	Thyroid hormones (T^4) TSH	7
Inuit infants	Perinatal PCBs/pesticides DDE ~ 1.2 ppm HCB ~ 0.1 ppm	Milk fat	↑	Incidence of *Otitis media*	11

Conclusions

Neonatal harbor seals show a number of responses that may be related to low-level perinatal exposure to dioxin-like PCBs and related compounds. These include reduced *in vitro* immune responses, serum thyroid hormone and retinol (vitamin A) levels, and high rates of infection. While preliminary, these data are consistent with the results of other studies demonstrating similar effects of PCBs, dioxins, and pesticides in human infants, laboratory animals, captive harbor seals, and free-ranging dolphins. The parallel findings observed in neonatal harbor seals and human infants underscore the need for future investigative research and suggest that wildlife models may be useful in elucidating the subtle health risks these compounds may pose to the unborn, newborn, and developing child.

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