Ethoxyresorufin-O-deethylase (EROD) Activity in Placental Tissue from Inuit Women Exposed to Organochlorines through the Arctic Food Chain

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1. Introduction

The Inuit living in Nunavik (Arctic Québec) receive an unusually high dose of various organochlorines due to their large consumption of traditional foods, most notably sea mammal fat (beluga, seals). Results of 105 breast milk analysis conducted in 1989-1990 showed a mean polychlorinated biphenyl (PCB) concentration of 2.9 mg/kg lipids (expressed as Aroclor 1260), a level five-fold greater than that measured in breast milk samples of women living in the southern part of the province (0.52 mg/kg lipids)[1]. Mean concentrations of chlorinated pesticides in milk samples from Inuit women were between three to five times greater than those of the reference population[2]. The difference between Inuit adults and southern Québec adults with regard to the mean dioxin-like compound concentration in plasma lipids was even more important (184 ng/kg vs 26 ng/kg)[3].

Several organochlorines have been identified as endocrine disrupting agents. Some (i.e. o,p'-DDT, p,p'-DDE) act directly through binding to steroid hormone receptors[4,5]. Others (i.e. dioxin-like compounds) operate through a variety of mechanisms, one of them being the alteration of hormone metabolism through induction of cytochrome P-450 dependent enzymes[6]. Newborns from Yu-Cheng mothers who were exposed *in utero* to both PCBs and PCDFs were smaller at birth than newborns from unexposed mothers. Correlation analysis revealed a statistically significant inverse relationship between aryl hydrocarbon hydroxylase (AHH) activity and weight of the newborn[7]. Hence P450IA1 associated enzyme activities may represent a valid biomarker of adverse developmental effects. This study aims at quantifying the activity of ethoxyresorufin-O-deethylase (EROD), a cytochrome P450IA1 associated enzyme, in placental tissue samples obtained from Inuit women living in Nunavik and control women from the Sept-Îles region with a low dietary exposure to organochlorines.

2. Methods

In Spring 1995, during the course of an ongoing cord blood surveillance program, 22 Inuit women giving birth at the Kuujjuaq regional hospital were asked to participate to this pilot biomarker study; all agreed and signed a consent form. A short questionnaire was administered to document physical characteristics, reproductive history and lifestyle habits (smoking). Information regarding the newborn

health status at birth (weight, size, head circumference and APGAR score) was extracted from the medical files. In order to effect comparisons, 20 non native women representative of the general southern Quebec population were recruited at the Sept-Îles regional hospital. Again all 20 women solicited agreed to join this study. The same consent form, questionnaire and tissue sampling procedures were used at both Kuujjuaq and Sept-Îles.

Cord blood samples were collected in 10-ml blue-lavender vacutainers (EDTA as the anticoagulant), centrifuged, and the plasma transferred in glass vials pre-washed with hexane. Determination of major PCB congeners and chlorinated pesticides in blood plasma was usually carried out on a 2-ml aliquot. The analytical steps were saponification with NaOH, extraction with hexane-ether (9:1) and chromatography on Florisil with hexane elution. Identification was effected by capillary gas chromatography (DB-5 column; J&W Scientific) with electron capture detection. Fourteen PCB congeners (IUPAC no 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) and 11 pesticides (aldrin, β-BHC, α- and γ-chlordane, *cis*-nonachlor, p,p'-DDE, p,p'-DDT, hexachlorobenzene, mirex, oxychlordane and trans-nonachlor) were measured. Detection limits were 0.03 mg/L for p,p'-DDT and β-BHC and 0.02 mg/L for other toxicants. Total blood lipids were also measured in order to express organochlorine concentrations on a lipid basis.

Meconium from the first stool was collected from the diaper, transferred into a conical tube and kept frozen at -20°C until analysis. Cotinine was extracted from meconium according to the procedure developed by Lewis[8]. Meconium samples were homogenized to a fine powder in glacial acetic acid and acetone. The solvents were evaporated and the sample dissolved in phosphate buffer. Cotinine was separated by solid phase extraction and measured by mass spectrometry.

Placentas were collected within one hour from expulsion. Connective tissue and blood vessels were removed and 10 grams samples were cut from the pink portion of the tissue. Samples were placed in polycarbonate vials and frozen at -80°C until shipping to the laboratory on dry ice. They were first pulverized in liquid nitrogen with a pestle and mortar to obtain a fine powder. For microsomes preparation, a 3 g aliquot was weighed in a pre-cooled 20 ml vial (dry ice). Microsomes were prepared by differential centrifugation according to a modification of Falzon et al. protocol[9]. Briefly, 2.5 ml of ice-cold 50 mM Tris pH 8.0-0.25 M sucrose was added to each vial prior to disruption of cells by a 15 sec treatment with a polytron. The suspension was transferred in a 15 ml tube and centrifuge at 10 000 x g for 20 minutes at 4°C. The supernatant was transferred in an Optiseal™ (13 x 48 mm, Beckman 361621). The pellet was re-suspended in 2 ml of Tris-sucrose and re-centrifuge at 10,000 x g for 20 min. The second supernatant was pooled with the first one. Microsomes were collected by centrifugation at 100,000 x g for 1h in a Beckman IL-100X centrifuge equipped with a TLA100.4 rotor. The pellet was re-suspended in 0.5 ml of 0.5 M KCl-Tris and homogenized with a "Dounce". The microsomes were aliquoted in 80 ul fractions and frozen at -80°C until further analysis. EROD activity was determined in placenta samples according to the microfluorometric method of Kennedy et al[10].

3. Results and discussion

Table 1 presents the mean concentrations for organochlorines detected in more than 50% of cord plasma samples for both groups. Levels measured in the Inuit samples were approximately 3 to 6 times greater than those for the control group ($p < 0.05$), with the exception of PCB congener 101 for which the mean concentration was higher in the Sept-Îles group ($p < 0.05$).

The mean EROD activity (arithmetic) for the Inuit group appeared greater than that for the control group, although this difference was not statistically significant (Table 1). Geometric means were 16 pmol/mg protein/min for the Inuit group and 4 pmol/mg/min for the control group. This apparent difference may be due to the greater proportion of smokers in the Inuit group, since smoking is known to induce EROD activity in placenta[11]. Nineteen out of the 22 Inuit women enrolled in the study smoked during pregnancy, compared to only 8 among the 20 women from Sept-Îles. Hence, further comparisons between non smokers from Nunavik and Sept-Îles could not be effected, because only three Inuit women did not smoke during pregnancy.

Table 1: Organochlorine concentration in cord plasma and EROD activity in placenta from Inuit women (Nunavik) and control women (Sept-Îles)

	Nunavik			Control group (Sept-Îles)		
	Mean[a]	95-% CI	N	Mean	95-% CI	N
Organochlorine[b]						
PCB 99	26	21-31	20	7	6-8	20
PCB 101	12	10-14	20	16	14-18	20
PCB 118	23	18-28	20	7	6-8	20
PCB 138	81	57-105	20	14	12-16	20
PCB 153	117	78-156	20	20	16-23	20
PCB 180	48	28-68	20	8	6-10	20
p,p'-DDE	504	378-630	20	110	91-129	20
HCB	71	53-89	20	11	10-13	20
Smoking[c]	28	12-44	19	19	1-37	8
EROD activity[d]	25	11-39	22	9	1-17	20

a Arithmetic mean and 95-% confidence interval
b Concentration in µg/kg lipids.
c Number of cigarettes consumed per day during pregnancy; 86% of Inuit women smoked compared to 40% in controls.
d pmol resorufin/mg protein/ minute

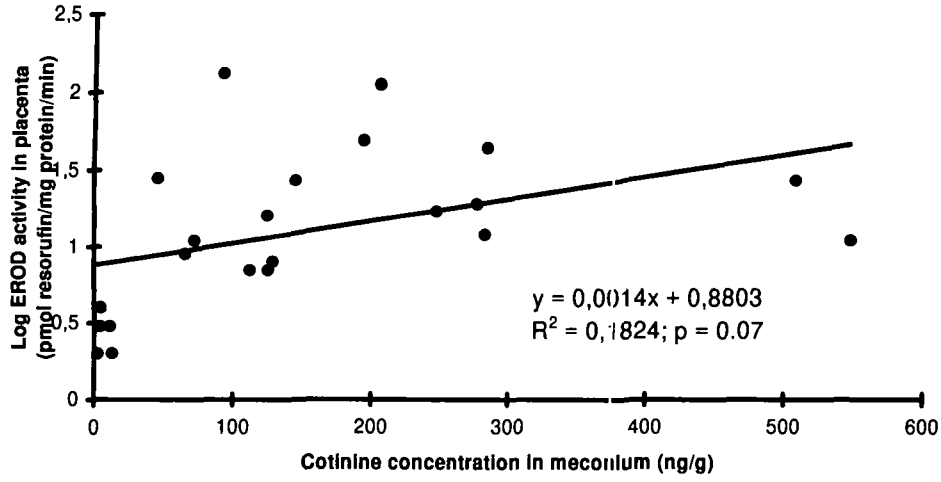

Figure 1: Relationship between the concentration of cotinine in meconium and EROD activity determined in placental microsomes from 22 Inuit women.

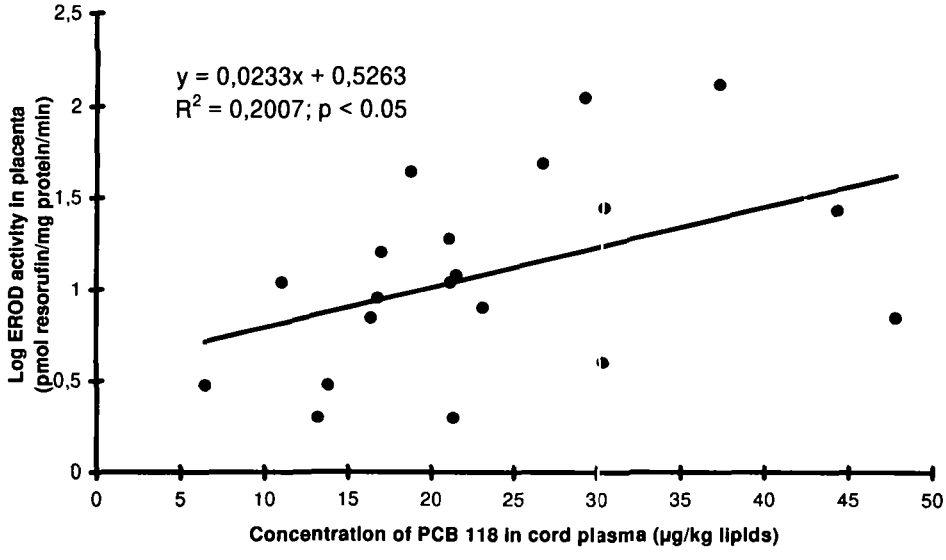

Figure 2: Relationship between PCB congener 118 concentration in cord plasma and EROD activity determined in placental microsomes from 20 Inuit women.

The relationship between placental EROD activity (log values) and organochlorine concentration in cord plasma or smoking were studied using simple linear correlation analysis. Results from these analysis should be interpreted with caution, because sample sizes are small and distribution of variables related to smoking may not meet the normality assumption. Only smoking, as depicted by number of cigarettes per day or cotinine concentration in meconium, was associated with placental EROD activity among controls. Using the number of cigarettes smoked per day as the smoking index, a R^2 value equal to 0.80 was obtained ($p<0.05$; data not shown). Figure 1 shows the weak relationship observed for the Inuit group between placental EROD activity and cotinine concentration in meconium. No association was noted with declared cigarette consumption. Interestingly, the concentration of PCB congener 118 in cord plasma also showed a weak but statistically significant association with EROD activity in the Inuit group. Similar relationships were observed between EROD activity and PCB 153 or DDE concentrations. A multiple linear regression analysis model with the concentration of cotinine in meconium and PCB 118 concentration in cord plasma as dependent variables explained that 33% ($p<0.05$) of EROD activity variance.

4. Conclusion

EROD activity in placental tissue obtained from Inuit women was roughly four-fold greater than that of a control group from Sept-Îles. Smoking as well as organochlorine exposure seemed to modulate placental EROD activity in the Inuit group. Whether or not this induction is associated with adverse developmental effects in the Inuit population is presently being investigated. Statistical analysis of results obtained with other biomarkers in placental tissue (DNA adducts, stress proteins) and cord blood lymphocytes (sister chromatid exchanges, immunological parameters) are in progress.

5. Acknowledgments

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6. References

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