Polar Bear Hepatic Cytochrome P450 1A1 and 1A2: Immunoquantitation, EROD/PROD Activity and PCB Levels

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1. Introduction

Contamination of the arctic ecosystem by anthropogenic compounds has resulted in exposure of high trophic level mammals such as polar bear (*Ursus maritimus*) to lipophilic xenobiotic contaminants accumulated through the food web. The polar bear is an ideal species for monitoring organochlorine (OC) exposure as its distribution is circumpolar and its diet consists almost entirely of ringed seal (*Phoca hispida*) blubber[1]. Exposure to polychlorinated aromatic compounds such as PCBs induces the mixed function oxidase (MFO) system cytochrome P450, a ubiquitous enzyme group common to mammals, birds, fish and microorganisms. Cytochrome P450 enzymes subsequently catalyze oxidative metabolism of xenobiotic and endogenous substrates. Total PCB concentrations as high as 70 μg per gram lipid have been reported in polar bear liver[2]. Ringed seals from the same geographic location have PCB levels six to fifteen times lower and apparent threshold levels below this have been linked to reproductive failure and other physiological abnormalities in marine mammals[3]. The polar bear therefore may be subject to greater potential toxicological risks from PCB exposure than other arctic mammals.

We have recently characterized the hepatic cytochrome P450 system of free-ranging polar bears immunochemically[4]. Antibodies prepared against purified rat cytochromes P450 1A1, 1A2, 2B1 and 3A1 cross-reacted with polar bear isozymes demonstrating immunochemical relatedness between the species and the potential for development of immunoassay biomarkers. The 1A1, 1A2 and 2B1 homologues appeared to be induced, probably due, in part, to exposure to PCBs. Densitometric quantitation of hepatic microsomal P450 1A1, 1A2 and 2B1 protein levels, catalytic EROD and PROD activities and organochlorine and $MeSO_2$-metabolite levels have been determined for 13 adult male polar bears. In this study, we report on the relationships between cytochrome P450 1A1 and 1A2 protein levels, EROD/PROD

activities and PCB levels to explore the influence of PCBs on enzyme induction and potential use of immunoassay quantitation as a bio-indicator of organochlorine exposure in polar bear.

2. Methods

Liver samples. Liver samples from 13 adult male polar bears, aged 5 to 22 years, were collected in a controlled hunt in April 1992 in the Canadian arctic near Resolute, Northwest Territories. All bears had been exposed to the tranquilizing drug, Telazol®, from 0.5 to 11 days prior to death. Liver samples were removed by biologists within 10-15 min. after death and frozen at -196°C in liquid nitrogen. Hepatic microsomal fractions were prepared and suspended in 0.25 M sucrose and stored at -80°C until needed.

Antibody preparation. Primary antibodies against rat cytochromes P450 were generated in female New Zealand rabbits immunized with electrophoretically homogeneous proteins. Polyclonal (poly specific) antisera IgG P450 1A1 recognizing both 1A1 and 1A2 forms was prepared. Immunoglobulin G (IgG) was purified from a pool of heat-inactivated antisera. Secondary antibodies used were horseradish peroxidase (HRP) conjugated goat anti-rabbit $F(ab')_2$ IgG.

Immunoblotting and quantitation. Separation of hepatic microsomal protein (10 µg/well) by SDS-PAGE was performed as described elsewhere[4]. Resolved proteins were electrophoretically transferred to nitrocellulose and probed with antibodies. Protein bands were revealed by HRP oxidation of 4-Cl-1-naphthol which formed a coloured product. Based on the results of monoclonal antibody rat cytochrome P450 1A1, we chose the band with the faster electrophoretic migration to be 1A1. The levels of cytochrome P450 1A1 and 1A2 homologous protein in polar bear microsomes were quantitated by densitometric scanning of the immunoreactive bands on the blots using a Visage® 110 video bio-imaging system, and comparison and normalization with purified rat cytochrome P450 standards. Polar bear 1A1 and 1A2 protein levels deduced from the immunoblots represented relative rather than absolute values as the strength at which rat generated antibodies recognized polar bear isozymes may have differed.

Catalytic activities. Ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-depentylase (PROD) activities were measured using a spectrofluorometric kinetic assay. The fluorescent resorufin content was quantitated in each sample from a standard curve using known amounts of resorufin.

Chemical analysis. Extraction and separation of organochlorines from 0.5 grams of homogenized liver sample was accomplished by the method of Letcher *et al.*[5]. PCB congeners were determined using a recently developed method for determination of organochlorines in a single fraction using gas chromatography coupled with mass spectrometry in the electron impact mode (GC/EI-MS) and a characterized polar bear fat extract as a secondary standard[6]. The extracts were initially spiked with ^{13}C-labelled CB-28, CB-52, CB-118, CB-153, CB-180 and CB-194 standards. Recoveries of the ^{13}C-labelled PCBs were determined using a PCB-154 performance internal standard. Recoveries were always greater than 85%. The PCBs were identified and quantitated using a Hewlett-Packard 5987B GC/EI-MS at 70 eV using a DB-5 column (30 m, 0.25 mm i.d.) under the following conditions: initial temperature held for 3 min. at 100°C, 20°C/min. to 180°C and 5°C/min. to 320°C. The injector, transfer line and ion source temperatures were 250°C, 300°C and 200°C, respectively[6]. There are 15 congeners which accumulate in polar bears[2]. The sum of congeners (S-PCB) is reported.

3. Results and Discussion

Densitometric quantitation revealed the cross-reacting hepatic P450 1A1 protein levels to be close to ten times higher than 1A2 (Table 1). The di-ortho, non-coplanar PCB congeners, 99, 138, 153, 180 and 170/190 accounted for ~90% of S-PCBs. These congeners are structurally consistent with PB-type inducers (i.e. P450 2B) in laboratory rodents[7], and are not expected to be P450 1A inducers in the polar bear. Nevertheless, concentration of S-PCBs may correlate with concentrations of P450 1A inducing non-ortho PCB congeners, such as 126, which has not yet been determined. The most active P450 1A inducing mono-ortho congener, PCB-105, is undetectable in polar bears. PCB-118, which is frequently an important contributor to TCDD toxic equivalents because of its relatively high concentration, is < 1% of S-PCBs in polar bear, significantly lower than in most animals[2]. However, two relatively active mono-ortho congeners, 156 and 157 are present at 1-2% of S-PCBs[2].

Varying residual concentrations of the tranquilizer, Zolazepam, a component of the tranquilizing drug Telazol®, were found in liver (Table 2). Zolazepam appeared to be an inducer of P450 2B1 (not shown). P450 1A1 and 1A2 protein levels showed no significant relationship with Zolazepam levels in liver. Both P450 1A1 (r^2=0.78) and 1A2 (r^2=0.73) (Figure 1a) protein levels were strongly correlated with S-PCBs (Table 2) and appeared to have zero-intercepts, suggesting P450 1A-type enzymes in polar bear were induced by PCBs or by other MC-type inducers that correlate with S-PCBs. Mixed MC- and PB-type P450 induction by di-ortho PCBs has been demonstrated in rat hepatic microsomes[7]. We are presently reanalyzing these livers for strong MC-type inducers such as PCDDs, PCDFs and non-ortho PCBs, although the levels are expected to be very low. Polar bear liver has been shown to contain 2-23 ng/kg of TCDD and OCDD[8].

Catalytic EROD activity increased with increasing cytochrome P450 1A1 and 1A2 protein levels to a maximum value of 1500 pmol/min/mg microsomal protein at P450 1A1 concentrations of 1.0 pmol/mg protein and P450 1A2 concentrations of 7.5 pmol/mg protein (Figures 1b and c). Correlation between EROD and P450 protein levels is much improved if Bears E and H, which had the highest P450 protein and PCB levels, are considered as outliers. It is possible that catalytic activity in Bear E and H liver samples was not as well preserved as in the other samples. However, there was no indication of any higher levels of P420 protein in these two samples than the others, which would be indicative of inactive protein. Furthermore, the large amounts of hemoglobin contamination observed in the microsomes of all bears was not disporportionately different for bears E and H[4]. It is possible that maximal catalytic activity is dictated by other limiting factors than P450 protein concentrations. Reduced activity of cytochrome C (P450) reductase has been observed for high P450 1A1, low EROD active beluga liver microsomes[9] and may explain the lower EROD activity observed here. NADPH cytochrome P450 reductase is an essential electron-transport enzyme precursor for cytochrome P450 monooxygenase activity[7]. The relationship between P450 1A1 and 1A2 protein levels and PROD activity were similar to EROD. PROD is catalyzed primarily, but not exclusively, by P450 2B forms in rat and beluga liver[9] which suggests that catalytic PROD activity may be primarily 1A-type in polar bear. In conclusion, densitometric quantitation of P450 1A1 and 1A2 immunoblots correlated well with S-PCB levels. Quantitative immunodetection should be an effective bio-indicator of polar bear PCB and organochlorine exposure for polar bear, and ultimately an index of arctic ecosystem health.

Table 1. Microsomal cytochrome P450 protein levels and catalytic activity for individual adult male polar bear livers*

	P450 protein** (pmol/mg micro. protein)		Catalytic Activity*** (pmol/min/mg micro. protein)	
Polar Bear	P450-1A1	P450-1A2	EROD	PROD
J	1.1 (0.2)	7.7 (1.4)	1571 (65)	24.5 (3.5)
B	0.7 (0.4)	3.9 (1.0)	192	n.d.
E	3.8 (0.4)	28.1 (6.3)	1459 (341)	24.5 (3.5)
L	1.0 (0.2)	8.2 (0.7)	1680 (394)	28.0 (0.0)
F	0.9 (0.0)	7.1 (1.3)	794 (170)	17.5 (3.5)
I	1.2 (0.4)	8.4 (2.6)	1138 (272)	24.5 (3.5)
C	1.4 (0.2)	14.3 (1.4)	1266 (176)	24.5 (3.5)
A	1.6 (0.1)	14.7 (1.0)	1401 (147)	21.0 (7.0)
G	1.4 (0.4)	11.1 (1.1)	1477 (177)	31.5 (3.5)
K	0.8	6.0 (0.7)	1056 (253)	21.0 (0.0)
D	0.7 (0.4)	5.3 (1.0)	1013 (95)	25.0 (11.0)
H	2.8 (0.0)	25.1 (3.5)	1596 (140)	28.0 (0.0)
M	0.5 (0.2)	4.7 (1.6)	863 (210)	17.5 (3.5)

* ordered top to bottom, those bears most likely feeding recenting to those most likely fasting
** mean of densiometrically quantitated immnuoblots, n=3 except for 1A2 (n=4)
*** two duplicate measurements (n=4) at periods ~1 month apart (bear B one dulpicate, n=2)
standard deviation in bracketts

Table 2. S-PCB and residual Zolazepam levels for individual adult male polar bear livers*

Polar Bear	S-PCBs (ug/g, lipid wt.)	Residual Zolazepam (ng/ml)
J	15.9	3
B	13.5	0
E	42.2	18
L	9.8	9
F	23.7	8
I	17.6	9
C	17.4	3
A	24	21
G	26.3	42
K	13.8	21
D	2.3	3
H	30	2
M	11.4	7

* ordered top to bottom, bears most likely feeding recenting to those most likely fasting

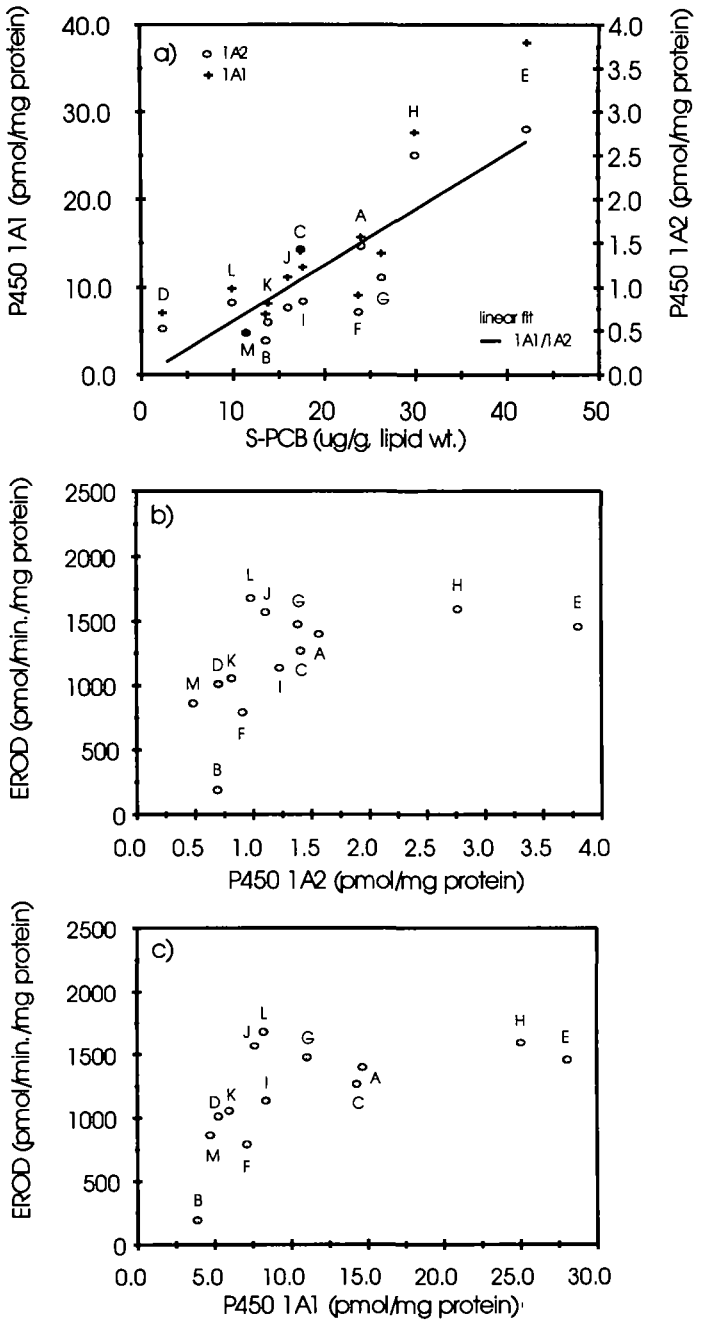

Figure 1. Relationships between a) S-PCB and 1A1/1A2 protein levels for polar bear hepatic microsomes b) 1A2 protein levels and EROD activity and c) 1A1 protein levels and EROD activity.

4. References

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