Cytochrome P450 mediated enzyme activities in relation to congener specific PCB accumulation in arctic seals

J. Wolkers[*], I.C. Burkow[**], R.F. Witkamp[***], C. Lydersen[****], S. Dahle[*], and M. Monshouwer[***]

[*]Akvaplan-niva, N-9005 Tromsø, Norway

[**]Norwegian Institute for Air Research, N-9005, Tromsø, Norway

[***]Department of Veterinary Basic Sciences, Division of Pharmacology, Pharmacy, and Toxicology, Utrecht University, P.O. Box 80.175, NL-3508 TD Utrecht, The Netherlands

[****]Norwegian Polar Institute, N-9005 Tromsø, Norway

Introduction

The arctic ecosystem is exposed to a range of organic contaminants of which PCB's are of particular importance because of their abundance and adverse biological effects. Predatory arctic mammals are particularly susceptible to pollution due to their large seasonal lipid cycles, resulting in a release of accumulated contaminants and an additional acute exposure during periods of fat mobilization (1).

The cytochrome P450 (CYP) enzyme system plays a prominent role in the first (oxidative) step of contaminant metabolism. Some metabolites arising from these reactions have been shown to be particularly harmful (2), making the CYP enzymes important mediators in many contaminant induced biological effects in animals. Large species differences exist in the presence and activity of CYP isoforms, resulting in differences in contaminant loads, accumulation patterns and biological effects between species, even if the exposure is similar. Therefore, knowledge about CYP is not only essential for understanding the load, fate and potential toxicity of contaminants, but also for using it as a biomarker in different species.

Although the important function of CYP in contaminant metabolism and contaminant toxicity is undisputed, these enzymes have received relatively little attention in marine mammals. This paper presents a study of the presence of different hepatic CYP isoforms in arctic seals. Further, CYP activities were related to PCB burdens, and the PCB accumulation pattern in seals relative to the PCB pattern in their food. Finally, the use of CYP as a biomarker for PCB exposure in seals was briefly evaluated.

Material and Methods

Sub-adult ringed seals (*Phoca hispida*) and harp seals (*Phoca groenlandica*) were sampled along the coast of Svalbard, Norway in May and August respectively. Liver and blubber were sampled and body length, body mass, and total blubber mass (3) were assessed. Congener specific PCB analyses (4) were carried out in seals (blubber) and their main food items. Microsomal ethoxyresorufin-O-deethylation (EROD), caffeine N-3 demethylation, and 6β-testosterone hydroxylation activities were measured with and without selective CYP inhibitors as described previously (5). Alpha-naphthoflavone and furafylline were used as CYP1A and CYP1A2 inhibitors, respectively (6,7). Ketoconazole was used as selective CYP3A inhibitor (8). Western blots were performed using monoclonal anti-bodies raised against rat CYP1A, CYP2B1, and CYP3A to detect microsomal CYP proteins. To avoid differences in PCB concentrations due to fluctuations in total blubber mass the PCB body burden (μg PCB/kg body weight), was calculated.

Results and Discussion

CYP activities and PCB burden

CYP activities in the harp seals were particularly high as compared to ringed seals (Table 1), but also to seals from other areas. Harp and hooded seals (*Cystophora cristata*) obtained from the east coast of Greenland had 20 and 2 times lower EROD respectively (9).

Table 1. Hepatic cytochrome P450 activities (mean ± sd) in $pmol \cdot min^{-1} \cdot mg\ protein^{-1}$ of ringed and harp seals. Specified are the activities of caffeine N-3 demethylation (CAF), ethoxyresorufin-O-deethylation (EROD), and testosterone 6β-hydroxylation (TST).

Cytochrome P450 activities	Ringed seals (n = 14)	Harp seals (n = 13)
CAF	213 ± 90	516 ± 155
EROD	65 ± 51	449 ± 146
TST	459 ± 337	952 ± 319

Although differences due to species can not be completely excluded, CYP induction due to contaminant exposure may also play an important role. Surprisingly, the average total PCB burdens were only 130 μg/kg body weight in the harp seals from the present study as compared to 200 μg/kg body weight in the ringed seals. Multiple regression analyses revealed a strong positive relation between PCB's with a maximum of 1 *ortho*-Cl (CYP1A inducers), and EROD (r^2 adjusted = 68 %) in the ringed seals. This relation was completely absent in the harp seals. Exposure to other contaminants may have resulted in these high CYP activities, but also dietary factors can have played a role. The summer diet of harp seals consists mainly of crustaceans (10), having a high carotene content. Carotenes have been shown to be potent CYP inducers, even at low dosages (11).

Inhibition characteristics

The substrates and the inhibitors used were chosen based on their relative selectivity towards different CYP isoforms in laboratory animals and humans. Likewise, the antibodies used were raised against specific rat CYP enzymes. The results from this study therefore provide only indirect evidence for the presence of particular CYP isoforms. Consequently, extrapolation towards seals must be made with some caution. Table 2 shows the effect of three inhibitors on the CYP activities in ringed and harp seals.

Table 2. Inhibition of caffeine N-3 demethylation activity (CAF), ethoxyresorufin-O-deethylation (EROD), and testosterone 6β hydroxylation activity (TST) in ringed seal (RS) and harp seal (HS) microsomes by α-naphthoflavone (5 μM), furafylline (5 μM), and ketoconazole (5 μM). Values are expressed as a percentage of the control activity (100 %).

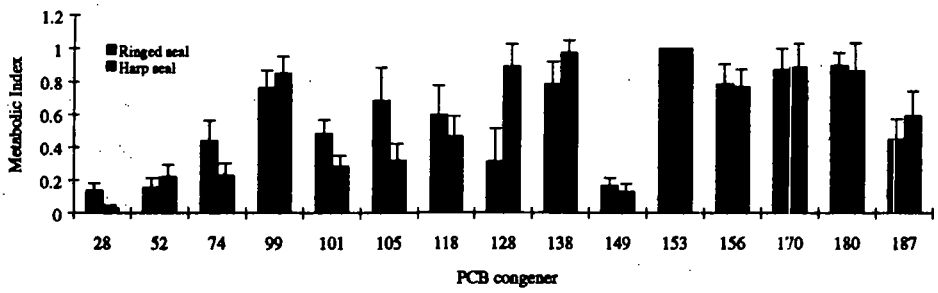
Inhibitor	control	α-naphthoflavone		Furafylline		Ketoconazole	
		RS	HS	RS	HS	RS	HS
CAF	100	26±1	21±10	95±1	77±20	35±17	95±4
EROD	100	<1	<1	105±4	93±13	39±5	58±5
TST	100	75±9	76± 4	99±3	95±9	12±8	22±3

Alpha-naphthoflavone was the only effective inhibitor of both EROD and the caffeine N-3 demethylation activity in both species, supposed to represent CYP1A1 and CYP1A2 activity respectively. This suggests that only one CYP1A isoform is present in the seals, which was supported by the Western blots. Only one protein cross-reacting with rat CYP1A antibodies was found. Testosterone hydroxylation involves different CYP enzymes. Formation of 6β-hydroxytestosterone in laboratory mammals is mediated by CYP3A (8, 12). Only ketoconazole resulted in a substantial reduction in the formation of 6β-hydroxytestosterone, suggesting that this activity is CYP3A mediated. The Western blot analyses confirmed the presence of CYP3A. Two clear protein bands cross reacted with rat anti-CYP3A antibodies. In addition one band cross reacted with rat anti-CYP2B antibodies (data not shown). However, since the formation of the testosterone 16β metabolite, characteristic for CYP2B activity (13), was extremely low in both species, the functional significance of this isoform is unclear.

Metabolic capacity

The metabolic index (MI), calculated as the ratio between the relative contribution of each PCB congener (PCB X / PCB 153) in seal blubber and in food, for ringed and harp seal is shown in fig. 1. MI indicates if the congener accumulates more (index > 1) or less (index < 1) than PCB 153 (14), while a low MI suggests metabolism. Congeners with vicinal H-atoms at *o,m* positions and a maximum of 1 *o*-Cl (PCB's 28, 74, 105, 118), supposed to be metabolized by CYP1A-like isoforms, or congeners with vicinal H-atoms at *m,p* positions and 2 or more *o*-Cl's (PCB's 52, 101, 149), metabolized by CYP2B and 3A-like isoforms, showed values well below 1 in most cases. This indicates metabolism. On the other hand, congeners with no vicinal H-atoms at the *o,m*- or the *m,p*- positions (PCB's 153, 180, 187) and congeners with no vicinal H-atoms at *m,p* positions and 2 or more *o*-Cl's (PCB's 99, 128, 138, 170) considered persistent, had in general a high MI's, indicating slower metabolism or even accumulation. With a few exceptions the MI patterns in the two seal species were similar and corresponded well with the different CYP isoforms.

Fig. 1. Metabolic index in harp and ringed seals per PCB congener. Values are expressed as the ratio between the relative presence in seals and in their food, (PCB X / PCB 153) *seal* / (PCB X / PCB153) *food.*



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