Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part C 145 (2007) 256–264
CBP
www.elsevier.com/locate/cbpc

Cytochrome P450 1A expression and organochlorine contaminants in harbour seals (*Phoca vitulina*): Evaluating a biopsy approach

Marta G.L. Assunção [a,b], Kelsey A. Miller [a,c], Neil J. Dangerfield [a], Stelvio M. Bandiera [c], Peter S. Ross [a,*]

[a] *Marine Environmental Quality Section, Institute of Ocean Sciences, Fisheries and Oceans Canada, P.O. Box 6000, 9860 West Saanich Road, Sidney, BC, Canada V8L 4B2*
[b] *ICBAS — Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal*
[c] *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada*

Received 29 September 2006; received in revised form 22 December 2006; accepted 28 December 2006
Available online 12 January 2007

Abstract

We previously reported *in vivo* induction of cytochrome P450 1A (CYP1A) by β-naphthoflavone in skin and liver biopsies of captive harbour seals (*Phoca vitulina richardsi*). The present study evaluated CYP1A expression (immunoblot analysis and ethoxyresorufin *O*-deethylase activity-EROD) in harbour seals using two study designs: i) skin and liver biopsies from 20 harbour seal pups captured from coastal British Columbia (BC, Canada) and temporarily housed in captivity; and ii) skin biopsies from 42 free-ranging harbour seals captured and sampled on-site in multiple locations in BC and Washington State (USA). Toxic Equivalency Quotients (TEQs) were calculated for polychlorinated biphenyl, polychlorinated dibenzo-*p*-dioxin, and polychlorinated dibenzofuran residues measured in blubber from a subset of study animals ($n=30$). CYP1A data from the seal pups held temporarily in captivity show that CYP1A protein levels were greater in liver than skin and that CYP1A protein and EROD activity were correlated in skin and liver. However, analysis of free-ranging seals from different sites revealed that blubber organochlorine TEQ values did not correlate with skin CYP1A levels. CYP1A protein levels and EROD activities in skin of seal pups from the BC locations and from Puget Sound were relatively low, possibly reflecting contaminant levels that were not high enough to elicit a response, a small sample size, or methodological limitations. Our results show that CYP1A measurements in skin show promise as a biomarker of contaminant exposure, but that refinements to techniques and a larger sample size are needed.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Biomarker; Biopsies; Cytochrome P450 1A; Environmental contaminants; Harbour seal

1. Introduction

The harbour seal (*Phoca vitulina*) can serve as a 'sentinel species' of marine ecosystem contamination, because of its wide distribution and its role as a top predator in marine food webs (Ross, 2000). Marine mammals occupying a high trophic level in the marine food web accumulate high concentrations of lipophilic environmental contaminants. Organochlorine contaminants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) are routinely detected in free-ranging harbour seals from British Columbia (Canada) and Washington State (USA) (Addison et al., 2005; Ross et al., 2004) and are capable of producing adverse endocrine, reproductive and immunological effects in both pinniped and cetacean species (Martineau et al., 1994; Ross et al., 1996; Ross and Troisi, 2001). The assessment of contaminant levels and health endpoints in free-ranging populations of marine mammals is therefore of continuing conservation interest.

Induction of hepatic cytochrome P450 1A (CYP1A) represents a well-established biomarker of exposure to planar halogenated and polycyclic aromatic hydrocarbons in aquatic vertebrates, including marine mammals. Hepatic CYP1A is a member of the cytochrome P450 superfamily of enzymes involved in Phase I oxidative metabolism of exogenous compounds. The CYP1A subfamily in mammals includes two enzymes, CYP1A1 and CYP1A2 (Nerbert et al., 1991). Gene

* Corresponding author. Tel.: +1 250 363 6806; fax: +1 250 363 6807.
E-mail address: RossPe@pac.dfo-mpo.gc.ca (P.S. Ross).

1532-0456/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2006.12.015

cloning and sequencing studies revealed the presence of homologous CYP1A1 and CYP1A2 genes in liver of several seal species and confirmed the highly conserved structure of these proteins in pinnipeds (>90% amino acid sequences similarity) (Teramitsu et al., 2000; Tilley et al., 2002). The mechanism of CYP1A induction involves initial binding of planar PCBs, PCDDs, PCDFs and related compounds to the aryl hydrocarbon receptor (Hahn, 1998; Stegeman and Hahn, 1994), and is biologically linked to detrimental effects associated with exposure to these organochlorine contaminants (Safe, 1994). Induction of hepatic CYP1A, determined by measurement of catalytic activity (mainly ethoxyresorufin-*O*-deethylase (EROD)) and CYP1A protein, has been previously reported in phocid seals including harbour seals, harp seals (*Phoca groenlandica*), hooded seals (*Cystophora cristata*), ringed seals (*Phoca hispida*) and grey seals (*Halichoerus grypus*) (Addison and Brodie, 1984; Addison et al., 1986; Goksoyr, 1995; Wolkers et al., 2002, 1998). In addition, this induction has been correlated with organochlorine exposure in studies of pinnipeds. Hepatic CYP1A levels were significantly higher in seals living in polluted environments when compared to more pristine areas (Mattson et al., 1998; Nyman et al., 2000) and hepatic CYP1A levels have been positively correlated with contaminant burdens measured in seal blubber (Chiba et al., 2002).

Routine measurement of hepatic CYP1A expression in free-ranging marine mammals presents unique challenges. Relying on liver samples that occasionally become available from individual animals captured as fisheries by-catch, killed in controlled hunts or from stranded and recently deceased animals (Troisi and Mason, 1997), is often random and may not permit representative sampling. Non-lethal (minimally invasive) tissue sampling techniques such as skin or blubber biopsies represent an ethically-acceptable and potentially powerful approach that can be applied to free-ranging marine mammal populations (Ben-David et al., 2001; Fossi and Marsili, 1997; Miller et al., 2005). In addition, such an approach enables sequential analysis of the same population over a period of time, as well as the evaluation of communities at risk.

Although CYP enzymes are concentrated in liver, they are also found in extrahepatic tissues including skin (Bickers et al., 1986; Raunio et al., 1995). Studies with laboratory rodents demonstrated that cutaneous CYP1A is responsive to prototypical inducers such as 3-methylcholanthrene (3MC) and β-naphthoflavone (BNF) (Bickers et al., 1986; Khan et al., 1992). We recently demonstrated that CYP1A protein and EROD activity are measurable in skin biopsy samples obtained from captive harbour seals. Using orally-administered BNF, we were able to demonstrate the functional responsiveness of CYP1A in skin and liver to a known CYP1A inducer, thereby confirming that the regulation of CYP1A in the skin of marine mammals is similar to that in liver (Miller et al., 2005). In other studies, CYP1A expression was assessed in skin biopsies collected from marine mammals in the wild and in captivity using CYP1A-associated catalytic activities and immunohistochemical tissue staining (Ben-David et al., 2001; Fossi et al., 2000, 1997; Godard et al., 2004). Thus, CYP1A expression in skin biopsies represents a viable option to lethal liver sampling, as skin can be obtained relatively easily without unduly harming the animals being sampled. However, it is not known if CYP1A expression in skin accurately reflects organochlorine exposure, despite the fact that organochlorine contaminants in marine mammals tend to localize in blubber, which lies immediately below the dermis.

The purpose of the present study was to assess the use of skin as a surrogate tissue for liver. CYP1A protein and EROD activity were quantified in skin biopsies of free-ranging harbour seal pups and adults sampled from southern British Columbia and northern Washington State, two regions showing regional differences in industrial contaminant inputs. In addition, we examined the relationship between skin CYP1A levels and blubber organochlorine concentrations using Toxic Equivalency Quotients (TEQs) in a subset of the study animals.

2. Materials and methods

2.1. Reagents

Resorufin was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI, USA) and ethoxyresorufin from Sigma Chemical Company (St. Louis, MO, USA). Alkaline phosphatase-conjugated goat $F(ab')_2$ anti-rabbit IgG was purchased from Biosource International (Camarillo, CA, USA). Purified rat CYP1A1, purified rat CYP1A2, polyclonal rabbit anti-rat CYP1A2 antiserum (recognizing both rat CYP1A1 and CYP1A2) and liver microsomes from adult male Long Evan rats treated with 3-methylcholanthrene (3MC) (25 mg/kg/day × 4 days) were prepared as described previously (Chang et al., 2003; Ngui and Bandiera, 1999). The liver S9 fraction from a β-naphthoflavone (BNF)-treated harbour seal (50 mg/kg/day × 3 days) was obtained from our previous study (Miller et al., 2005).

2.2. Study animals

In August 2000, 20 Pacific harbour seal (*Phoca vitulina richardsi*) pups were live-captured in the Fraser River estuary (British Columbia, Canada — 49°10′N, 123°11′W—Fig. 1) using rapid deployment of a modified beach seine net, as described elsewhere (Jeffries et al., 1993). Details of handling and animal husbandry are reported elsewhere (Mos and Ross, 2002). Briefly, seal pups were weighed and transported in canine carrying cages to a temporary holding facility. Pups were sampled 2 days post-capture and were fasted during this two-day period. Liver and skin biopsies were obtained from the pups under general anaesthesia (isoflurane gas) by veterinary staff, as described elsewhere (Mos and Ross, 2002). Briefly, liver biopsies were taken through a 1 cm incision using a 14 gauge × 10 cm soft-tissue biopsy needle (EZ Core, Products Group International, Inc., Lyons, CO, USA). Skin and blubber biopsies were obtained from a small shaved area (clippers) on the lateral side of the body (anterior to the pelvis) with an 8 mm biopsy punch (Acuderm, Ft. Lauderdale, FL, USA). After skin biopsies were taken, xylocaine topical anaesthetic (Astra Pharma, Mississauga, ON, Canada) was applied. Tissue

Download English Version:

https://daneshyari.com/en/article/1978364

Download Persian Version:

https://daneshyari.com/article/1978364

Daneshyari.com