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Immune-relevant and new xenobiotic molecular biomarkers to assess anthropogenic stress in seals

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ABSTRACT

Harbour seals as top predators and indicators for ecosystem health are exposed to increasing pressure caused by anthropogenic activities in their marine environment. After their lactation period of about 24 days pups are weaned and left to hunt on their own. Little is known about the development of their immune system and a better understanding of anthropogenic impacts on the general health and immune system of harbour seal pups is needed. mRNA transcription of six immuno-relevant biomarkers was analysed in 13 abandoned harbour seal pups from the North Sea, fostered at the Seal Centre Friedrichskoog, Germany. RNAlater blood samples were taken at admission, day 22 and before release and analysed using RT-qPCR. Significant differences in HSP70, cytokine IL-2 and xenobiotic biomarkers AHR, ARNT and PPARα transcription were found between admission, during rehabilitation and before release. Highest levels at admission may result from dehydration, handling, transport and contaminant exposure via lactation. The significant decrease is linked to health improvement, feeding and adaptation. The increase before release is suspected to be due to infection pressure and contaminant exposure from feeding on fish. Molecular biomarkers are a sensitive tool to evaluate health and pollutant exposure and useful to serve as early warning indicators, monitoring and case-by-case tool for marine mammals in human care and the wild.

1. Introduction

Harbour seals are one of the two reproducing pinniped species in the North and Baltic Seas and, as top predators, may be important indicators for ecosystem health in these waters (Reddy et al., 2001). The North Sea is heavily influenced by multiple anthropogenic activities such as shipping, offshore construction, chemical pollution and eutrophication (Harwood, 2001; Waterman et al., 2003; Vinther and Larsen, 2004). Compounds like persistent organic pollutants (POPs) are considered hazardous (Wolff et al., 2010) and relevant contaminant concentrations have been found in various organs of pinnipeds (Law et al., 1991; O'Shea, 1999; Das et al., 2003), mainly being accumulated in liver and blood in harbour seals (Ahrens et al., 2009; Weijs et al., 2009). POPs are known to negatively affect the immune system and have been reported to result in increased susceptibility to viral infections in seals (De Swart et al., 1996; Ross et al., 1996). In 1988/89 and 2002 Phocine Distemper Virus (PDV) caused the death of 40–60% of the harbour seal population in the North Sea (Müller et al., 2004; Härkönen et al., 2006). The massive decline has been discussed as being related to a debilitated immune system caused by immunotoxic contaminants (Brouwer et al., 1989; Ross et al., 1996).

After their short lactation period of about 24 days pups are weaned abruptly and left to hunt on their own (Muelbert and Bowen, 1993). Little is known about the development of the immune system in harbour seal pups after weaning and about the influence of contaminants, stress and infectious diseases upon their health status. A better understanding of the early biological effects of anthropogenic impacts on the cellular level is needed. Contaminants associated with immunotoxicity in marine mammals are suspected of being mediated among others via the aryl hydrocarbon receptor (AHR), with "dioxin-like" compounds having the greatest immunotoxic potential (Luster, 1987; Safe, 1990).

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AHR up-regulation is activated by environmental pollutants such as PCDDs, PCBs and PCDFs and one pathway depending on the bioactivity and chemical structure of the compound. Together with its dimerisation partner, aryl hydrocarbon receptor nuclear translocator (ARNT), it is responsible for DNA binding and dimerisation (Beischlag et al., 2008; Fujii-Kuriyama and Kawajiri, 2010; Chopra and Schrenk, 2011).

Peroxisome proliferator-activated receptor (PPARα) is a nuclear receptor belonging to the xenobiotic metabolism and involved in the induction of detoxifying enzymes, regulation of mRNA transcription and functions as a transcription factor (van Raalte et al., 2004). PPAR alpha, AHR and ARNT have been used to assess pollutant-induced changes and the ecological risk of dioxin-susceptibility in tissues of mammals (Kim et al., 2002, 2005; Asakawa et al., 2008). Cytokines are important cell mediators and responsible for the initiation, amplification and maintenance, of an immune response (Mosmann and Sad, 1996). IL-2 and IL-10 are used as standard interleukins in applied research and known to be correlated with inflammatory disease in marine mammals (Fonfara et al., 2008; Beineke et al., 2007). Heat shock proteins are essential to measuring stress as well as immune reactions as they are involved in the cellular response to viral, bacterial and parasitic pathogens (Lindquist, 1986; Chen and Cao, 2010). The aim of this study is to establish immuno-relevant biomarkers using mRNA analyses as a tool for assessing the health status of marine mammals in human care as well as to serve as early warning indicators for threats to marine mammal populations examined in the wild. Three markers of the xenobiotic metabolism (ARNT, AHR & PPARα) were established for the first time in blood samples of pinnipeds using RT-qPCR. Cytokines (IL-2 & IL-10) and heat shock protein (HSP70) were examined as markers for stress and inflammatory disease. This study takes advantage of the unique opportunity to closely monitor harbour seal pups during rehabilitation and measured mRNA transcription levels of pollutant-induced and immuno-relevant biomarkers as early and sensitive parameters to reflect biological effects of anthropogenic stress on the cellular level.

2. Material and methods

2.1. Blood sampling

Abandoned harbour seal pups (13; males $n = 5$; females $n = 8$), found in the Wadden Sea area of Schleswig-Holstein in 2011 and fostered at the Seal Centre Friedrichskoog, Germany, were investigated. Blood samples were taken from the epidural intravertebral vein, as described by Dierauf and Gulland (2001), at admission in June 2011, on day 22 of rehabilitation and before the seals' release back into the North Sea. Seals were restrained manually while blood was taken. At admission seals were between two and nine days old, determined by their weight, size, navel and canine development. 22 days passed between the first and second measurement. 80–140 days passed between first and third examinations and about 60–120 days passed between second and third examinations (Table 1).

For mRNA isolation, EDTA blood collection tubes were prepared with RNAlater (RiboPure-Blood Kit, Ambion Life Technologies).

2.2. Blood status

Venous whole blood was collected in tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant. Blood status was generated with a ScilVet ABC™ Animal Blood Counter (Scil Animal Care Company GmbH, D-68519 Viernheim, Germany).

Standard haematology profiles, including white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), platelets (PLT), lymphocytes (LYM), monocytes (MO) and granulocytes (GRA) were taken at admission, day 22 and before release.

Antibiotics, anti-inflammatory drugs, anti-emetics and antacids were given depending on individual fitness after admission until 23–54 days before final examination. The compensatory milk product Immulak (Vet Concept) and a small, increasing amount of North Sea herring were given from admission. By day 20 the diet consisted of North Sea herring exclusively.

After reaching a weight of 15 kg and a standard haematology profile seals were moved from an enclosure with 7–9 animals to a larger enclosure with 20–30 animals before release.

2.3. Real time RT-PCR analysis

EDTA blood samples were taken in EDTA monovettes containing 3 ml of RNAlater and ~1 ml full blood. The samples were ice-cooled until arrival at the lab where RNA was isolated directly or samples were frozen at −20 °C until isolation. Total RNA was isolated from 500 to 700 μL EDTA blood (RiboPure™-Blood Kit, Ambion Europe, Huntington, UK) according to the manufacturer's protocol.

RNA concentration was determined using 2 μl RNA in a Thermo Scientific Nanodrop 2000 unit (Peqlab Biotechnologie GmbH) and 80–100 ng/μL RNA was reverse transcribed with murine reverse transcriptase (RT-PCRCore Kit™; Applied Biosystems, Weiterstadt, Germany) under the following temperature conditions: 8 min at 25 °C, 15 min at 42 °C, 5 min at 96 °C and at 4 °C until samples were removed. cDNA was stored at −20 °C and served as template

Table 1
Sex, estimated age in days and weight in kg at admission, day 22, and final examination in investigated harbour seals ($n = 13$) in 2011.

Pv #	Sex	Admission	Age (days)	Weight (kg)	Day 22	Age (days)	Weight (kg)	Final examination	Age (days)	Weight (kg)
Pv 1	F	10.06.	2	11.2	01.07.	24	11.8	25.08.	82	32.2
Pv 2	F	10.06.	7	9.8	01.07.	29	11.0	17.10.	136	34.2
Pv 3	F	17.06.	2	7.6	08.07.	31	8.8	04.09.	96	28.6
Pv 4	F	12.06.	7	7.0	03.07.	29	8.6	07.09.	101	25.9
Pv 5	M	16.06.	2	11.4	07.07.	24	12.4	09.08.	100	26.6
Pv 6	M	10.06.	2	9.0	01.07.	24	9.6	25.08.	83	31.2
Pv 7	M	15.06.	9	7.6	06.07.	31	10.4	07.09.	99	31.2
Pv 8	M	16.06.	9	7.6	07.07.	25	9.6	25.08	87	29.0
Pv 9	F	12.06.	N/A	8.2	03.07.	~22	9.4	25.08.	~91	26.2
Pv 10	F	10.06.	4	9.0	01.07.	25	10.4	18.10.	126	35.2
Pv 11	F	17.06.	9	8.0	/	/	/	/	/	/
Pv 12	F	15.06.	8	8.4	/	/	/	/	/	/
Pv 13	M	15.06.	8	10.8	/	/	/	/	/	/