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Acute toxic responses of the rockfish (*Sebastes schlegeli*) to Iranian heavy crude oil: Feeding disrupts the biotransformation and innate immune systems

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ABSTRACT

To clarify the toxic effects of Iranian Heavy Crude Oil (IHCO) from the "*Hebei spirit*" oil spill, innate immune toxic effects defending on biotransformation pathway have been investigated on fish exposed to IHCO. Juvenile rockfish were exposed to IHCO in gelatin capsules by feeding. The effects on multiple fish biotransformation enzymes (Cytochrome P4501A and glutathione-*S*-transferase) and the expression level of the several immune response genes, including interleukin-1beta, granulocyte colony-stimulating factor and Cathepsin L, were measured in the liver, spleen and kidney. The tissue-specific expression patterns of these genes demonstrated that the highest expression levels of Cytochrome P4501A, glutathione-*S*-transferase, interleukin-1beta, granulocyte colony-stimulating factor, interferon stimulated gene 15 and Cathepsin L were found in the liver and that the TNF receptor was high in spleen. The oil-fed fish had significantly higher concentrations of biliary fluorescent metabolites and Cytochrome P4501A expression during the initial stage (12 ~ 48 h after exposure) than those in the liver and kidney of the sham group. Similarly, the highest mRNA expression levels of interleukin-1beta and granulocyte colony-stimulating factor were detected in the liver at the early stages of exposure (12 h after exposure). Following exposure, the levels of interferon stimulated gene 15 and granulocyte colony-stimulating factor mRNA remained high at 120 h after exposure in the liver but the levels of interleukin-1beta and Cathepsin L gradually decreased to an expression level equal to or less than the sham group. Our data suggest that the innate immune and hepatodetoxification responses in oil-fed fish were induced at the initial stage of exposure to the IHCO at the same time but several immune-related genes decreased to less than that of the sham group after the initial stage of response. Therefore, immune disturbances in fish exposed to IHCO may allow the pathogens, including the infectious diseases, to more easily affect the oil exposed fish.

1. Introduction

The *Hebei Spirit* oil spill occurred in 7 December 2007 was one of the largest tanker spills in recent years, comparable to the *Prestige* oil spill in 2002 and the *Tasman Spirit* in 2003 [1]. The nearshore region was seriously impacted by the spill or cleanup effort during the first year after the spill. In our previous study, more than five of the fish species monitored after the spill exhibited evidence of exposure and effects of petroleum, including the induction of cytochrome P450 (CYP1A) and genetic damage in heavily contaminated sites. Although concentrations of tissue and water parent polycyclic aromatic hydrocarbons (PAHs) were no longer elevated above levels at a reference site by 3 months after the spill, biliary PAH metabolites and CYP1A activity remained elevated for the first couple of years and fluctuated thereafter until now [2–7]. Their persistence most likely reflects exposure to the persistent sediment-bound contamination, most likely through continuous ingestion of prey, which includes contaminated benthic organisms. It can be expected that the biliary PAH metabolites and indices of CYP1A induction will remain elevated in these fish as long as their prey continues to be contaminated by sediment-bound PAHs. The persistent residual oil in marine environment is known to induce disturbances in the immune system of fish [8,9]. It is well known that individual PAHs have led to the disruption of the hepatic detoxification and immune system in fish [10–12]. However, the immunological mechanism of toxicity and the relationship between the hepatic detoxification and

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immune responses has not been fully investigated in oil contaminated fish. In addition, crude oil is a massive complex mixture of PAHs, and the complicated toxic reactions from these individual chemicals are not understood.

Fish from several PAH-contaminated regions of the USA and Canada have been reported to exhibit epizootics of hemic, neural, connective tissue, gonad, and liver cancers [13]. Additionally, the fish from Taean exhibited a high incidence of skin disease (more than 30%) during the three years after the spill incident when compared with the populations from an uncontaminated site (Unpublished Data, 2009). These reports may suggest an increase in the potential risk of cancer or other diseases after oil exposure at a spill site.

The innate immune response is considered to be an attractive effect-based monitoring tool due to its capacity to predict population disturbances due to the modification of disease susceptibility [14]. Innate immunity acts as the host's first line of defense against pathogen invasion, whereas adaptive, acquired, and specific immunity plays a vital role in protection. However, the effects of crude oil on the innate immune system of fish is not well documented and depend on how the hydrocarbons are taken up, metabolized, and excreted by the organism.

In this study, to clarify the effects of crude oil spilled by the *Hebei Spirit* on fish's hepatic detoxification and the immune system, multiple hepatic detoxification enzymes and cytokines, such as tumor necrosis factor receptor (TNFr), interleukin-1beta (IL-1β), interferon stimulated gene 15 (ISG15), granulocyte colony-stimulating factor (G-CSF) and Cathepsin L (CTSL), were evaluated in juvenile Rockfish. TNF-α is a member of the TNF superfamily and mainly acts as a pro-inflammatory cytokine that is important in both immune response inflammation and host defenses. Multiple factors from bacteria, viruses, and parasites stimulate TNF-α production in the host [15–17]. Other cytokines and regulatory molecules evaluated in the current study include IL-1β, which is mainly produced by macrophages and has been characterized in fish. Cathepsins are lysosomal cysteine proteases of the papain family. CTSL plays a physiological role in the initiation of protein degradation as an important lysosomal cysteine protease, which is involved in antigen presentation [18], parasitic infection [19]. G-CSF is central to the neutrophil-based immune defenses due to its regulatory role in the growth, differentiation, survival, and activation of neutrophils and their precursors.

The released and residue oil may be readily absorbed by many routes of exposure and can pass directly through the plasma membrane and results in lethal and sub-lethal effects through the metabolic pathway and disruption of immune system. In this study, we demonstrated the effects of crude oil exposure on the hepatic detoxification system. Therefore, whether absorbed crude oil-induced has an effect on the innate immune defense system was studied.

2. Materials and methods

2.1. Fish and exposure

Two-year-old juvenile rockfish (*Sebastes schlegeli*) weighing 180 ± 2.0 g (mean ± S.D.) and 20 ± 2.0 cm in length were purchased from a local fish farm in Geoje, Korea. Fish were acclimated for two weeks in flowing seawater at 16 °C on a 16 h light/8 h dark photoperiod in the laboratory prior to the beginning of experiments to make sure they were disease free. Water quality variables, including pH, salinity and dissolved oxygen were monitored daily.

To study crude oil exposure, fish were exposed orally. Rockfish were individually fed gelatin-capsulated Iranian Heavy Crude Oil (IHCO) using a needleless syringe. The exposure level in fish is shown in Table 1. Sham fish were fed gelatin-capsulated corn oil. Nine fishes were sampled at 0, 12, 24, 48, and 120 h after exposure. Before they were killed by a blow on the head, length, weight, sex and gonad weights were measured. Samples of brain tissue, spleen, gall bladders and liver were frozen in liquid nitrogen and stored at −80 °C until analysis.

Condition factor [CF = total body weight (g) × 100/length^3 (cm)], hepatosomatic index [HSI = liver weight (g) × 100/total body weight (g)] and gonadosomatic index [GSI = gonad weight (g) × 100/total body weight (g)] were calculated for all samples.

2.2. Bile fluorescence analysis

Synchronous scanning of fluorescent bile metabolites, focusing on 1-hydroxypyrene, was used as a rough indicator of fish exposure to PAH [16,20]. Frozen gall bladders were disrupted to release the bile. The tissue mass was centrifuged briefly to sediment unwanted tissue and the bile was analyzed as described by Jung et al. [2,3,21]. 1-Hydroxypyrene (Aldrich, Louis, USA) was used as a standard at concentrations (in 50% ethanol), ranging between 50 and 500 nM.

2.3. Quantitative real-time PCR analysis

Total RNA was extracted from rockfish liver, spleen and heart by Isogen (Wako, Japan). The purified total RNA was reverse transcribed into cDNA using the First-strand cDNA synthesis kit (Invitrogen, CA, USA). The RT-PCR assays for gene transcripts in rockfish tissue were performed using a two-step procedure. The PCR primers are shown in Table 1. The β-actin gene was used as a

Table 1
Oligonucleotide primers used in this study.

Gene	Accession number	Product size (bp)	Primer 5′-3′	PCR conditions
CYP1A1	AAU81558	207	Fw : TCATGACCTGTTTGGAGCTG Rv : AAGGATCTCCAGGATGAAGG	30″ at 95 °C, 30″ at 60 °C, 1′ at 72 °C
G-CSF	AB490455	209	Fw : ATCAGGACGACTTGGACGGAGT Rv : TCAAACGATGATCTCAGTGTGG	30″ at 95 °C, 30″ at 57 °C, 1′ at 72 °C
CTSL	AB491141	201	Fw : AGTATGTCAAGGACAACCAG Rv : TGTCGTTGACAGAGTTGTACG	30″ at 95 °C, 30″ at 57 °C, 1′ at 72 °C
ISG15B	AG72218	122	Fw : GCACAAGGACACTTTCATCATG Rv : CCACTTTCTGCTGGATCAAGAC	30″ at 95 °C, 30″ at 60 °C, 1′ at 72 °C
GST	AAV40975	198	Fw : GAAGAACCTGCAGGGCTACA Rv : GTCAGGCCCTCAAACATGCG	30″ at 95 °C, 30″ at 58 °C, 1′ at 72 °C
TNF receptor	AB490889	128	Fw : ACTCCAACACCGTCTCTGCT Rv : GTCGCCTCCGTCTCTCAATA	30″ at 95 °C, 30″ at 60 °C, 1′ at 72 °C
IL-1β	AB491084	325	Fw : CAACCTCATCTCTTCGCCATG Rv : CAGAACTCTGGGTGTAGGGT	30″ at 95 °C, 30″ at 60 °C, 1′ at 72 °C