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CHARACTERISTIC FORMATION OF HYDROXYLATED PHENANTHRENE METABOLITES IN FISH (SEBASTES SCHLEGELII) ORGANS EXPOSED TO PHENANTHRENE

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Introduction

Recently, there have been frequent oil-spills along Korean coastal areas. In December 2007, about 10,800 tons of oil was spilled from the M/V Hebei spirit which polluted more than 375 km of the west coastal of the South Korea.[1] After the Hebei spirit accident and response operation for removal of the bulk oil, many researches and studies have been conducted to assess the impact of the oil spill on marine environment and ecosystems. Most of these studies have focused on polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs among the numerous oil originating contaminants because it is well known that PAH compounds have adverse biological effects on marine ecosystems.[2] However, when microorganisms are exposed to pollutants such as PAHs, xenobiotic enzymes are released in response and metabolites are formed through the processes of oxidation, reduction, hydrolysis and etc. However, the metabolites or transformed products of these parent compounds have not received much attention. Although the xenobiotic metabolism mechanism were activated to reduce effect of toxic compounds[3], PAH metabolites are known to be toxic as adducts to cellular macromolecules such as DNA, RNA, and proteins after enzymatic biotransformation. PAH toxicities such as mutagenesis, teratogenesis, and carcinogenesis, are highly structure dependent[3], and its metabolites have various types of isomers. Consequently, the toxicity of PAHs in the organism is highly related with isomeric structural transforming mechanism or metabolism such as Cytochromes P450 (CYP450) enzyme reaction. Different activated type of CYP450 under its super family species yield different distribution of transformed metabolites type, isomer ratios and absolute amounts.[3, 4] Therefore, several PAH exposure studies have been conducted to understand the characteristic metabolism by in vivo or vitro, however, varying metabolites formation results were obtained.[5, 6]

Most high molecular weight PAHs have “bay-region” and “K-region”, and phenanthrene (phen) is the simplest PAH that has both regions. Hydroxylated PAH metabolite isomers have different carcinogen potency according to Schmidt-Pullman electronic theory, especially the K-region epoxides.[7] Until now, most of phen exposure studies have focused on mammal tissues, urine, and sludge from wastewater, but to the best of our knowledge, no detailed metabolite formation pathway from fish exposed to phen has been done.

Therefore, in this study, the phen metabolites formation pathway was assessed by exposing the rock fish (Sebastes schlegelii) to phen. Additionally, the characteristic formation pattern in each fish organ such as bile, liver and muscle was investigated.

Materials and methods

Chemical and analytical method

An extraction procedure was developed in order to quantify phen, and the metabolites of phen (1-OH-phen, 2-OH-phen, 3-OH-phen, 4-OH-phen, and 9-OH-phen) in fish bile, liver, and muscle. The deuterated phen (phen-d9), and hydroxy-phen-d9 were used as an internal standard and the identification of the target analytes in the samples were analyzed using gas chromatography coupled with tandem mass spectrometry. The established method includes enzymatic hydrolysis, liquid-liquid extraction and derivatization for hydroxy PAHs.

Laboratory exposure design

Rock fish (Sebastes schlegelii) was exposed to phen by oral administration after anesthesia by 2-phenoxyethaol, and feed capsules were filled with dimethyl sulfoxide and methanol. Control group was exposed only to solvent, group 1 to 10 ppm, and group 2 to 30 ppm of phen by wt. A total of 46 fishes were introduced into each basin of exposure group and were not fed (decontamination period) for 2 weeks to reduce background metabolites concentration. Fish tissue samples were taken at 0 hr, 6 hr, 12 hr, and 29 hr for the control group, and 6 hr, 12 hr, 29 hr for groups 1 and 2. Tissue samples were stored in dry-ice box under -78.5°C and transported to the refrigerator under -80°C on the same day the samples were collected.

Results and discussion

Phen, exposed PAH compound, was detected in liver and muscle samples of experimental groups. Higher phen concentration was detected in the liver, followed by muscle but no bioaccumulation was observed in the liver or muscle from group 1 (Fig. 1, a). While the high phen concentration level of group 1 decreased with time, the group 2 remained unaltered. In contrast, a large increase of phen metabolites concentrations in the bile was observed in 12 hours in group 1 and an exponential increase was observed in 29 hours for group 2 (Fig. 1, c and d). Furthermore, an important difference between the two groups was that the highest phen metabolites concentration was observed at different exposure time for group 1 (12 hr) and group 2 (> 29 hr). Among the phen metabolites in the fish bile of all experimental groups, 3-OH-phen was the most dominant (67-82%), followed by 2-OH-phen (4-20%), 1-OH-phen (5-16%), 9-OH-phen (ND-4%), and 4-OH-phen (ND-3%) regardless of time and exposed concentration. This ratio was different from the results in previous in vivo or in vitro and human studies.[8, 9] It is known that PAH metabolite isomers have different carcinogen potency, especially, the K-region (9-, 10-) substituted PAHs metabolites are more toxic than L-region substituted.[7] Considering the result of metabolized hydroxy metabolite ratio in this study based on with "K-region" theory, Sebastes schlegelii may have less toxic metabolite producing pathway than other K-region predominant metabolite producing species such as rat.[4] The detailed discussion about the phen metabolites with various factors will be presented in Dioxin 2016.

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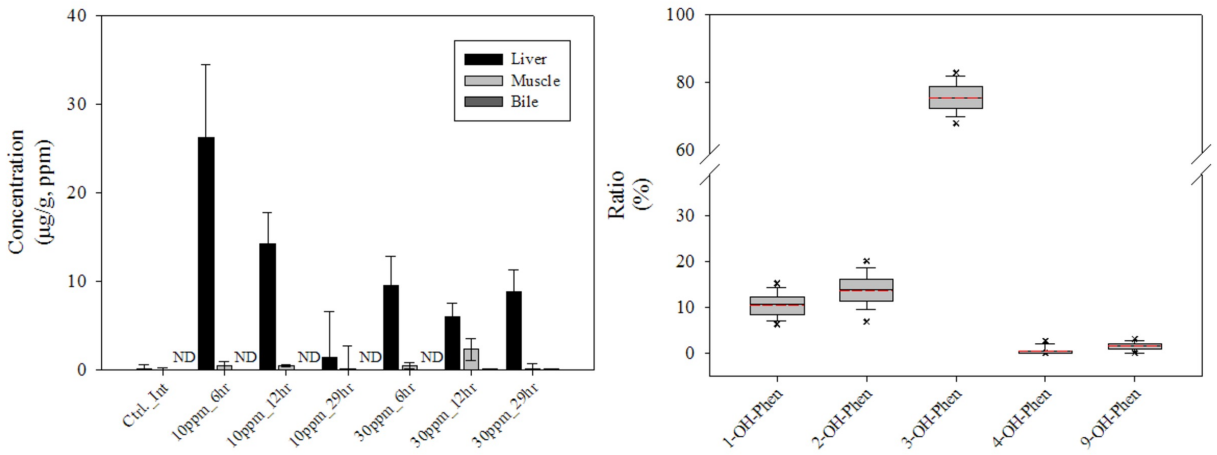

Figure 1 (a) Concentration of phen in liver, muscle and bile and sampling periods, (b) ratio of hydroxy phen isomers in bile of phen exposed group