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1-OH-Pyrene and 3-OH-Phenanthrene in Urine Show Good Relationship with their Parent Polycyclic Aromatic Hydrocarbons in Muscle in Dairy Cattle

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The toxicities of phenanthrene (PH) and pyrene (PY) are less than benzo(a)pyrene (BaP), but both compounds are found in higher concentrations in the air, feed, and food. Most PAHs are metabolized to hydroxylated compounds by the hepatic cytochrome P450 monooxigenases system. Metabolites are excreted into urine and feces. We determined concentrations of PH, PY and BaP in muscle and hydroxylated metabolites, 3-OH-PH, 1-OH-PY, and 3-OH-BaP, respectively, in urine from dairy cattle (n = 24). We also evaluated the relationship between parent compounds in muscle and their metabolites in urine. Concentrations of PH and PY in muscle ranged from 0.7~4.8 ng/g (1.8 ± 1.7) and 0.4~4.1 ng/g (1.2 ± 1.2), respectively. Concentrations of 3-OH-PH and 1-OH-PY in urine ranged from 0.1~5.9 ng/m*l* (2.9 ± 3.7) and 0.5~3.6 ng/m*l* (1.9 ± 2.3), respectively. Correlation coefficient for PY concentration in muscle versus 1-OH-PY in urine was 0.657 and for PH concentration in muscle versus 3-OH-PH in urine was 0.579. Coefficient determination for PY and PH concentrations in muscle was 0.886 and for 1-OH-PY and 3-OH-PH in urine was 0.834. This study suggests that 1-OH-PY and 3-OH-PH could be used as biomarkers for PAHs exposure in dairy cattle.

***Key words*:** Exposure biomarker, Polycyclic aromatic hydrocarbons, Dairy cattle, 1-OH-Pyrene, 3-OH-Phenanthrene

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous environmental contaminants present as mixtures in air and food (WHO, 1998). Phenanthrene (PH) and pyrene (PY) are found abundantly in the air and food, and thus could be used as an index of PAHs contamination (Costera *et al.*, 2009; Martorell *et al.*, 2010). Benzo(a)pyrine (BaP) is a known carcinogen in humans (Hecht *et al.*, 2010) and laboratory animals (Knafla *et al.*, 2006). The BaP is metabolized to an epoxide which binds covalently to macromolecules in body.

Metabolic activation of PAHs occurs primarily through the action of CYP1A family P450 monooxygenases, such as CYP1A1 which hydoxylates PAHs (Elovaara *et al.*, 2007; Vakharia *et al.*, 2001). Urinary monohydroxy-PAHs have been suggested as biomarkers for assessing human exposure to PAHs (Li *et al.*, 2008). Correlations were reported between PH concentration and the corresponding urinary monohydroxy-PH metabolites in people with airborne PH exposure (Chetiyanukornkul *et al.*, 2006), and between urinary PY and monohydroxy-PY metabolites in industrial workers (Fustinoni *et al.*, 2010; Liu *et al.*, 2010; McClean *et al.*, 2007). Urine 1-OH-PY concentrations were reported to be sensitive biomarker in rats orally exposed to PAHs for 28 d (Kang *et al.*, 2007). 1-OH-PY is the main PAH metabolite in human and goat, thus 1-OH-PY in urine and milk has been suggested as a marker of exposure in these species (Campo *et al.*, 2010; Onyemauwa *et al.*, 2009).

Though numerous studies evaluated 1-OH-PY and 3-OH-PH in humans, there have been few studies documenting concentrations of PAH metabolites urine from domestic animals how it relates to tissue PAH concentrations to our knowledge. The purpose of this study was to evaluate the correlations between parent compounds in muscle and their metabolites in urine by determination of amounts of BaP, PH, and PY in muscle and their respective hydroxylated metabolites, 3-OH-BaP, 3-OH-PH and 1-OH-PY in urine from dairy cattle.

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MATERIALS AND METHODS

Chemicals. Benzo(a)pyrene, phenanthrene and pyrene were purchased form Sigma-Aldrich (St. Louis, USA), and 3-OH-benzo(a)pyrene, 1-OH-pyrene and 3-OH-phenanthrene were purchased from Midwest Research Institute (Kansas, USA).

Sampling. Skeletal muscle and urine were randomly collected from same dairy cattle (n = 24) at slaughter houses in the Kyeunggi province, Republic of Korea.

Determination of parent compounds in muscle and their metabolites in urines. The concentrations of BaP, PH and PY in muscle and of 3-OH-BaP, 1-OH-PY, and 3-OH-PH in urine were determined using HPLC with fluorescence detection according to the method by Kang et al (Kang *et al.*, 2007). Briefly, 3 g of muscle frozen with liquid nitrogen, homogenized, extracted with 50 m*l* of hexane, and concentrated to about 1 m*l* at 45°C with a rotary evaporator. After purification using an activated Florisil cartridge (Waters, USA), PAHs were eluted with 18 m*l* of hexane and dichloromethane (3 : 1, v/v) solution. After drying the eluent at 45°C, it was dissolved with 1 m*l* of acetonitrile by sonication. For the determination of metabolites in urine, 7 m*l* of 0.2 mol sodium acetate buffer (pH 5.0) was added into 5 m*l* of urine for acidification and then β-glucuronidase (13,200 U) and sulfatase (220 U) solution were added. After incubating (37°C, 210 rpm) for 16 h and centrifugation (3,000 ×g for 10 min), the supernatant was purified with an activated Sep-Pak C_{18} cartridge (Waters, USA). The metabolites were eluted with 10 m*l* of hexane and dichloromethane (3 : 1, v/v) then evaporated at 45°C, and dissolved with 1 m*l* of acetonitrile.

Statistical analysis. Coefficient determination (R^2) were determined between the parent compounds and their corresponding metabolites by linear regression plotting by Microcal

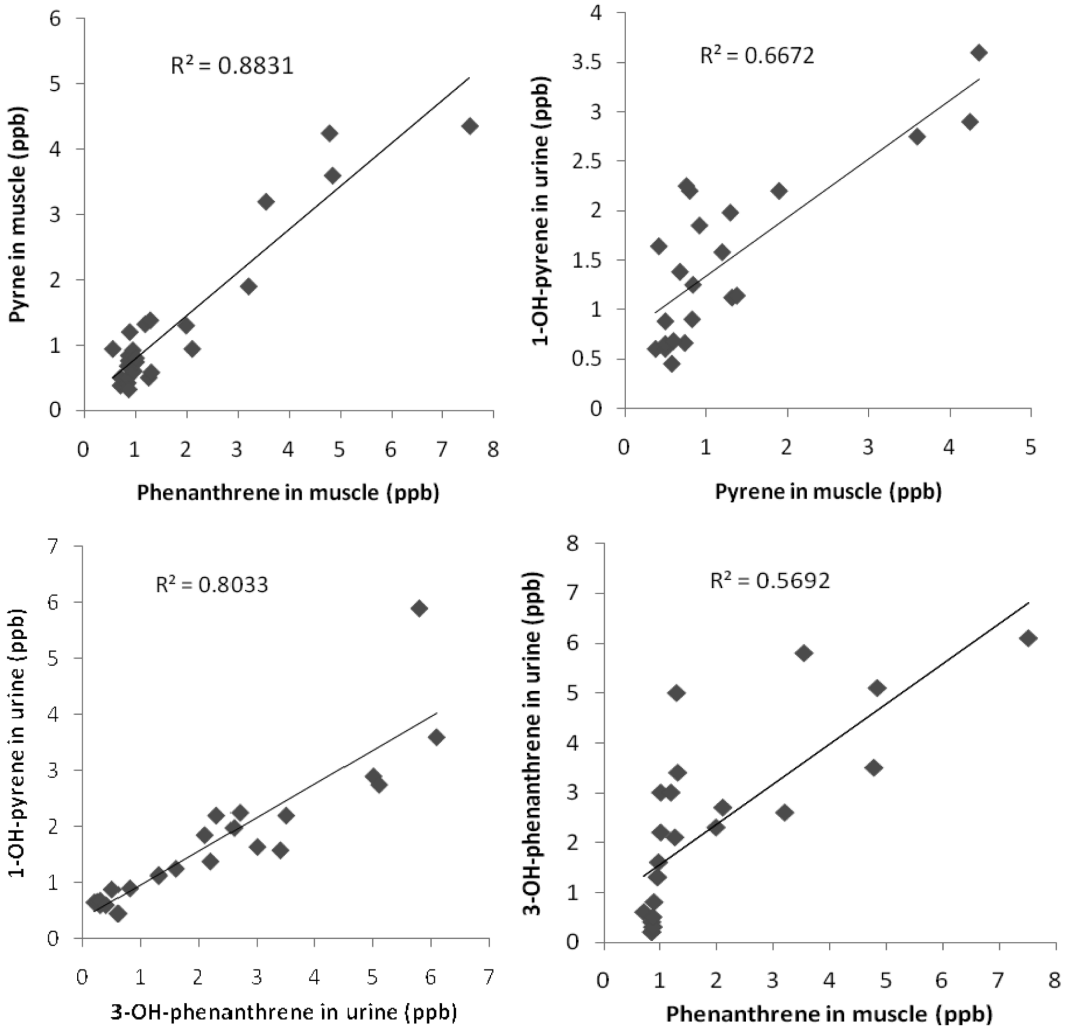

Fig. 1. Correlation between phenanthrene and pyrene in muscle and 3-OH-phenathrene and 1-OH-pyrene in urine of dairy cattle.

Table 1. Parent polycyclic aromatic hydrocarbon in muscle and their metabolites in urine in dairy cattle

	Muscle			Urine		
	Phenanthrene	Pyrene	Benzo(a)pyrene	3-OH-Phenanthrene	1-OH-Pyrene	3-OH-Benzo (a)pyrene
Mean ± SD	1.8 ± 1.7	1.2 ± 1.2	ND	2.9 ± 3.7	1.9 ± 2.3	ND
Range	0.7~4.8	0.4~4.1	ND	0.1~5.9	0.5~3.6	ND

Data are the ng/g in muscle and the concentrations of their metabolites are ng/m*l* in urine.
ND; not detected.

Origin 6.0 (Microsoft, USA).

RESULTS

Distribution of parent PAHs in muscle and their metabolites in urine. The concentrations of PH in muscle ranged from 0.7 to 4.8 ng/m*l* (mean = 1.8 ± 1.7). The concentrations of PY ranged from 0.4 to 4.1 ng/m*l* (mean = 1.2 ± 1.2). Concentrations of 3-OH-PH in urine were from 0.1 to 5.9 ng/m*l* (mean = 2.9 ± 3.7). The concentrations of 1-OH-PY ranged from 0.5 to 3.6 ng/m*l* (mean = 1.9 ± 2.3). Neither BaP in muscle nor its metabolite 3-OH-BaP in urine was detected.

Correlation between parent PAHs in muscle and their metabolites in urine. The coefficient determination (R^2) between the concentration of pyrene in muscle and 1-OH-PY in urine was 0.667, and for PH in muscle and 3-OH-PH in urine was 0.570. The coefficient determination for the concentration of PY and PH in muscle was 0.883 and for 1-OH-PY and 3-OH-PH in urine was 0.803.

DISCUSSION

Metabolites of PH, PY, and other PAHs in body fluid are useful biomarker for PAHs exposure in humans and other species (Gundel *et al.*, 2000; Jacob and Grimmer, 1996; Keimig *et al.*, 1983; Zhao *et al.*, 1992). The major hydroxylated metabolites for PY, PH and BaP are 1-OH-PY, 3-OH-PH and 3-OH-BaP, respectively (Jacob and Grimmer, 1996; Keimig *et al.*, 1983)

PY forms a relatively large proportion of the high molecular weight PAHs found in foods. Its metabolite, 1-OH-PY in urine is representative of occupational and dermal PY and total PAH exposure and it is a good indicator for mutagenic activity in animals and human liver (Buckley and Lioy, 1992; Jongeneelen *et al.*, 1988).

The main metabolite of PY in urine and milk is 1-OH-PY. Thus 1-OH PY could be considered as a marker of ruminant PAH exposure as it has been in humans. Because 1-OH-PY and 3-OH-PH were detected in milk, metabolites PAHs should be considered when evaluating milk safety (Lapole *et al.*, 2007).

3-OH-BaP and 3-OH-PH were most abundant metabolites of BaP and PH exposure, respectively, in human urine (Gundel *et al.*, 2000; Jongeneelen *et al.*, 1985). Urine can be obtained quickly and easily in live animals and people, and thus is a useful biological sample for assessment of exposure to environmental contaminants. Urine 1-OH-PY concentrations reflect recent exposure, while the presence of PAH-adducts reflects more persistent, long time exposure (Jongeneelen, 2001; Ovrebo *et al.*, 1994). The amount of the parent compound in tissues and urine are proportional to the dosage given in rats (Kang *et al*., 2009). Urinary 1-OH-PY could be used as a biological monitoring index for human exposure to high concentrations of PAHs, but it is not applicable for biological monitoring of extremely low concentration PAH exposures (Hara *et al.*, 1997). The major metabolite detected in goat urine and milk of goats orally dosed with PY, PH, and BaP was 1-OH-PY , thus it can be used as an indicator of the ruminant PAH exposure (Costera *et al.*, 2009). The geometric mean for 1-OH-PY in urine samples from people in the US was 0.050 ng/m*l* (Li *et al.*, 2008). The mean concentration of urinary 1-OH-PY in cattle from a remote rural area in Germany was 0.46~1.35 ng/m*l* and in cattle close to a busy highway was 3.27~10.5 ng/m*l* (Ferrari *et al.*, 2002). The findings of this study were similar. The concentrations of 1-OH-PY in urine from dairy cattle ranged from 0.5 to 3.6 ng/m*l*. These results suggest that dairy cattle are exposed to higher concentration of PAHs than people or that cattle metabolize PAHs differently. According to present study, levels of PH and PY in muscle and their metabolite in urine were also closely related to each other. This data also suggests that dairy cattle also are exposed to a similar ratio of PH and PY as people. Conclusively this study suggest that 1-OH-PY and 3-OH-PH are useful biomarkers for PAH exposure in dairy cattle as in people, but more extensive studies are required to use these biomarkers under conditions of low-level PAH exposure in dairy cattle.

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