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Identification of 1-hydroxypyrene in urine of dogs after pyrene injection and its excretion*

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Abstract—With high performance liquid chromatograph and fluorospectrometry, 1-hydroxypyrene is identified as a metabolite of pyrene in the urine of dogs after intramuscular injection of pyrene. When three successive doses of 1,2 and 5 μmol/kg wt. pyrene administered, urinary excretion of 1-hydroxypyrene reaches the peak level between 24th and 48th hours. Excreted 1-hydroxypyrene consists of only about 0.05% of injected pyrene.

Keywords: 1-hydroxypyrene; pyrene; polynuclear aromatic hydrocarbons.

INTRODUCTION

Polynuclear aromatic hydrocarbons(PAHs) represent an important group of pollutants among environmental carcinogens, and pyrene is one of the major components of PAHs in air, water and food. It is always found present in environmental PAHs mixtures (International Agency for Research on Cancer Polynuclear Aromatic Compounds, 1983). PAHs metabolites could be viewed as an index of biological absorption of PAHs from the environment. Urinary 10 hydroxypyrene was found as a major metabolite of pyrene in rats and rabbits (Boyland, 1964), in pigs (Keimig, 1983), and identified on in vitro incubation of microsome of human liver with pyrene (Jongeneelen, 1988). Urinary 1-hydroxypyrene was suggested recently for the assessment of human exposure to PAHs in the environment (Jongeneelen, 1988; Zhao, 1990). In this paper, urinary 1-hydroxypyrenen of dogs is identified after intramuscular injection of pyrene, and its excretion through urine investigated.

MATERIALS AND METHODS

Reagents

1-hydroxypyrene was synthesized in the laboratory and its purity was identified up to chromatographic grade by means of elemental analysis, infrared spectrometry, HPLC and GC/MS. Pyrene is analytical reagent grade, produced by Beijing Chemical Factory. Glucuronidase/aryl

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sulphatase, H-1 type, produced by Sigma, U.S.A. Methanol, analytical reagent, distilled before use.

Instruments

HITACHI HPLC model 638-50 used with the 650–10 lc fluorometric detector. HITACHI-MPF-4 Fluorospectrometer is used for scanning of fluorometric spectrum. SEP-PAK C18 cartridges is produced by WATERS, Japan.

Animal experiments

Two male dogs were habituated in the laboratory, weighing 17.5 kg and 15.5 kg respectively. Pyrene was dissolved in ethyl oleate as injective reagent (30 μmol/ml). Three doses were given to each animal by intramuscular injection successively at three-day period, and the dosages were 1, 2 and 5 μmol/kg wt. respectively. One day before the experiment, collection of dog urine was initiated, and the collection of all urine continued for 11 days. Time of sample collecting and sample volume were recorded, and urinary 1-hydroxypyrene was then determined.

Identification and determination of 1-hydrozypyrene in urine

Ten milliliters of urine sample was buffered with 5ml 0.5mol/L acetate buffer (pH 5.0), adjusted to pH 5.0 with 1.0mol/L HCl, and incubated for 1 h with 1000 U β-glucuronidase/aryl sulphatase at 37°C. A SEP-PAK C18 cartridge was used for the separation of the PAH metabolites. After priming at a rate of 8 ml/min., the sample was injected and the cartridge washed with 5 ml water. Retained solute was eluted using 8 ml methanol. The solvent was evaporated at 60°C under a constant flow of nitrogen to 0.5ml for HPLC analysis. Analysis was performed in a ODS silx-1 column (0.26×25m), column temperature is 20±3°C; mobile phase is 5 min. with 90% solvent A(60% bidist., 40% methanol), then a linear gradient to 90% solvent B(100% methanol) in 10 min., followed by 15min. 90% solvent B at 0.6 ml/min., injection volume: 30μl.

RESULTS AND DISCUSSION

Identification of urinary 1-hydrozypyrene

There is only one main peak on the chromatograph profile after the sample hydrolysed with enzyme, with the retention time being equal to that of the standard solution of 1-hydroxypyrene. In Fig.1, there is no significant peak in the chromatograph occurred for unhydrolysed urine. Collected fraction of the eluted peak was analysed with a fluorospectrometer. As compared with the standards of 1-hydroxypyrene (Fig.2), both have the same excitation and emission spectra, thus identifying the occurrence of 1-hydroxypyrene in the urine sample. Note that pyrene is not detected in a 10ml aliquot of dog urine (the detection limit for pyrene was 0.77 ng).

Excretion of urinary 1-hydrozypyrene after injection of pyrene into animals

After pyrene was injected into dogs, the quantity of excreted urinary 1-hydroxypyrene is not observed increased within 3 hours after the pyrene injection. From 6th hour, it starts to increase tremendously, reaching the maximum in 24–48 hours. As the injected amount of pyrene

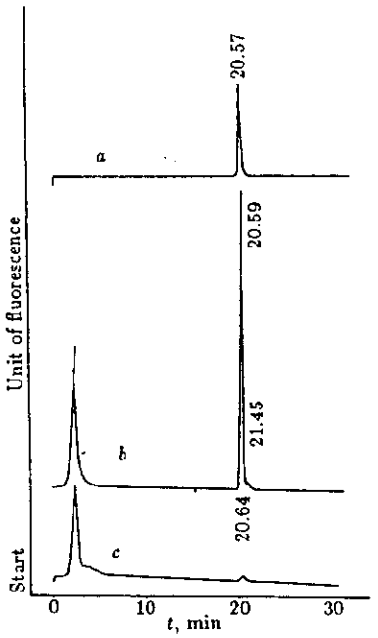

Fig.1 HPLC-chromatogram of urine of a dog after intramuscular injection 2 μmol of pyrene/kg wt.

a: the 1-OH-pyrene reference;

b: enzymic hydrolysis sample;

c: unhydrolysed sample.

increased, excretion of urinary 1-hydroxypyrene increases to a higher level correspondingly. Fig.3 shows the change of urinary 1-hydroxypyrene concentration with time. Fig.4 shows the quantitative excretion of 1-hydroxypyrene after intramuscular injections of different dosage. The results suggest that the excretion follows the same trend, even though significant variation exists between individual dogs.

It was shown from the excretion of two dogs administered with three doses that the amount of urinary 1-hydroxypyrene excreted is mainly in the interval from 24 to 48 hrs and it increases with the increase of administered amounts of pyrene. Excreted quantity of urinary 1-hydroxypyrene makes up a small portion of injected pyrene, about 0.04% and 0.06% for dog 1 and dog 2 respectively as indicated in Table 1.

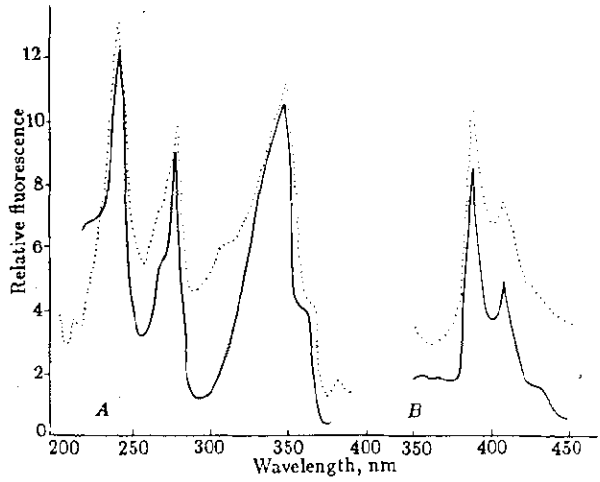

Fig.2 Fluorescence excitation (Fig 2a) and emission scans (Fig.2b) of the peak coinciding with the 1-OH-pyrene reference standard in the HPLC-chromatogram

..... the reference itself; — a dog urine sample

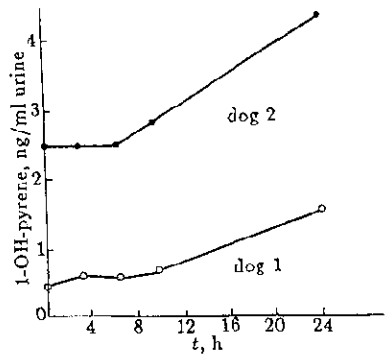

Fig.3 Changes of concentration of urinary 1-hydroxypyrene of dogs after intra muscular injection of 5μmol of pyrene/kg wt.

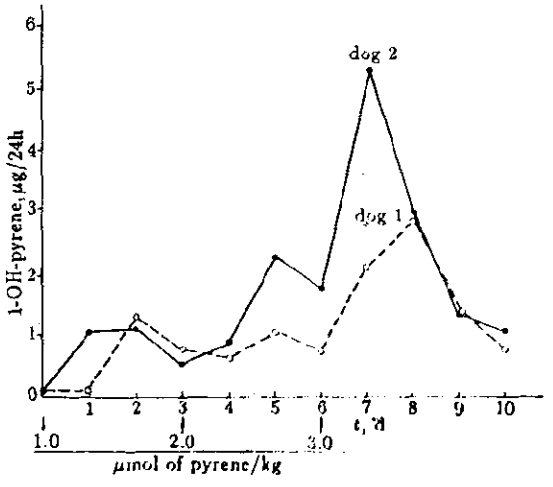

Fig.4 Excretion of 1-hydroxypyrene in urine of dogs after intramuscular injection of 1,2,5μmol of pyrene/kg wt (Arrows indicate the time of injection)

Table 1 Excreted amounts of 1-hydroxypyrene in urine of dogs after three successive intramuscular injections of pyrene

Dog No.	Administered pyrene dose, μmol/kg wt.	μmol of pyrene	1- Hydroxypyrene in urine μmol $\times10^{-2}$	ratio% * $\times10^{-2}$
1	1	17.5	0.94	5.4
	2	35.0	1.14	3.3
	5	87.5	2.98	3.4
sum	8	140	5.06	3.6
2	1	15.5	1.19	7.7
	2	31.0	2.29	7.4
	5	77.5	4.51	5.8
sum	8	124	7.99	6.4

* Excreted 1-hydroxypyrene/administered pyrene

This experimental work evidenced that urinary 1-hydroxypyrene is excreted after pyrene was administered intramuscularly to dogs, and the excretion rate increases as the amount of administered dosage of pyrene increased. No any other metabolite is detected. Therefore, 1-hydroxypyrene is the main metabolized substance after pyrene assimilated by dogs. It was reported that after oral administration of pyrene, excreted urinary 1-hydroxypyrene by rats was increased and showed a peak rate 24 hours after dose, then reducing to the normal level in 72 hours (Jongeneelen, 1985). About 10μg of 1- hydroxypyrene was recorded from the urine of rats when 500μg of pyrene was administered, and the excreted 1-hydroxypyrene occupied

1.85% of administered pyrene by molar amount, a higher ratio than that in our experiments with dogs(0.05% on the average), perhaps because rats have higher metabolic rate. In summary, urinary 1-hydroxypyrene has been evidenced in several animal experiments as the major end metabolized product either for rats, pigs or for dogs after pyrene is injected. In vitro test with microsome of human liver confirmed that only 1-hydroxypyrene was detected in considerable amount and no other metabolite appeared, and S_9 preparation from human liver and the Salmonella mutagenicity test illustrated that 1-hydroxypyrene could be a proper index as mutagenic activity of pre-mutagenic reagent of coal tar (Jongeneelen, 1988). Because of high sensitivity of urinary 1-hydroxypyrene, its determination is naturally presumed a good method for biological monitoring of human and animal exposure to the environmental pyrene.

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