Use of biomarkers in the assessment of exposure to polysyclic aromatic hydrocarbons

Proceedings: International Symposium of the Kanazawa University 21st-Century COE Program Vol. 2 (2004)
Hayakawa, K., Kizu, R., Kamata, N. (eds.)

ISSN 1348-3048

Use of Biomarkers in the Assessment of Exposure to Polycyclic Aromatic Hydrocarbons

Thaneeya CHETIYANUKORNKUL, Akira TORIBA,

Ryoichi KIZU and Hayakawa KAZUICHI

Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Ishikawa 920-0934, JAPAN

Abstract - Several polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and/or endocrine disrupting compounds produced through the combustion. Humans are exposed to PAHs via several routes from contaminated PAHs in the environment and as the resulting from their activities. To evaluate the amounts of PAH exposures in our body, HPLC determination of urinary hydroxy PAHs are useful as biomarkers to reflect the external exposures. By using this method, the exposure to PAHs in Japanese and Thai subjects were discussed.

I. Introduction

Several polycyclic aromatic hydrocarbons (PAHs) are toxic ubiquitous compounds, which are mainly formed through imperfect combustion of organic matters. Humans are daily exposed to PAHs from diesel exhaust particulate, cigarette smoke and many industrial processes. Human exposure to PAHs is a risk factor for the carcinogenicity as well as endocrine disruption, which is connected with their metabolism. To estimate the exposure amount of PAHs, it is therefore necessary not only to characterize distribution and occurrence of PAHs in the environment but also to quantify available and effective PAHs in human body because PAHs have multiple exposure routes. The biological monitoring of urinary metabolites of PAHs is useful to evaluate the exposure to PAHs, since PAHs are readily and predominantly metabolized to hydroxy PAHs (OHPAHs) as well as glucuronides and sulfates [1, 2]. Single urinary 1-hydroxypyrene was popularly used as a biomarker however it reflects only one parent PAH [2-4]. On the other hand, our data have reported that 2-hydroxyfluorene is more sensitive than 1-hydroxypyrene as a new biomarker of exposure to PAHs from smoking [2, 5]. Hence, determination of various OHPAHs as multiple biomarkers should provide more comprehensive profiles data of PAHs' exposure as an alternation.

Therefore, this work proposed urinary OHPAHs having 2-4 rings in human urine as useful multiple biomarkers. An HPLC-FL method for simultaneous determination of ten OHPAHs having 2-, 3- and 4-rings in urine has been developed [1]. Applications were examined in Japanese and Thai non-smoker subjects (rural villagers, red-taxi driver and traffic policemen).

II. Experimental

A. Developed HPLC-FL method

An HPLC simultaneous determination method of ten OHPAHs, 1-, and 2-hydroxynapthalenes (1- and 2-OHNaps), 2-hydroxyfluorene (2-OHFle), 1-, 2-, 3-, 4- and 9-hydroxyphenanthrenes (1-, 2-, 3-, 4- and 9-OHPhes), 3-hydroxyfluoranthene (3-Frt) and 1-hydroxypyrene (1-OHPyr) has been developed. The sample treatment involved enzymatic hydrolysis, following by the modification of double (Sep-Pak C_{18} and Silica) solid phase extraction, elution with hexane/ethyl acetate (9:1, v/v) and final separation on HPLC coupled with fluorescence detection. Deuterated 1-OHPyr was first synthesized by CYP450 and selected as a suitable internal standard. OHPAHs were separated well on

an alkylamide type reversed-phase column.

B. Applications

The developed method was applied to Japanese non-smokers who lived in Kanazawa city and Thai non-smokers who lived in Chiang Mai province (rural villagers, red-taxi drivers and traffic policemen). Their concentrations in both Japanese and Thai were compared.

III. Results and discussion

A. Developed HPLC-FL method

Ten OHPAHs were separated on an alkylamide-type reversed phase column, except for coeluted 1- and 9-OHPhes (Fig. 1). Deuterated 1-OHPyr was separated well prior to their non-deuterated. The developed method showed good linearity of calibration curves (r^2 ranged from 0.996 to 0.999). Intra and inter assays of urine samples treated enzymatically showed good reproducibility (RSD < 17%). The limits of detection (S/N = 3) were in the range from 0.05 to 32 μg/L.

B. Concentrations of urinary ten OHPAHs

Table 1 shows the urinary concentration levels of OHPAHs based on the unit of μmol/mol creatinine in Japanese and Thai subjects. In general, at least 6-9 of OHPAHs can be evaluated in both non-smoker and smoker groups. The mean concentration levels of OHNaps were higher than that of 2-OHFle, 1-OHPyr and OHPhes. The large scattering was found in OHNaps especially for 1-OHNap. This result was in agreed with previous observation [2, 5]. The concentrations of 1-OHPyr, 2-OHFle and 2-OHNap in Thais were quite higher than in Japanese.

Compare the urinary concentration (μmol/mol creatinine) to other countries, i) 1-OHPyr in rural villagers was close to that of asphat paver workers (1.35, preshift) [4], however higher than coke oven worker (0.89, preshift) in the Netherlands [6], ii) 1-OHPyr in non-smoker groups (all Thai groups) were higher than non-smokers groups in Germany (0.04), Italy (0.08), Sweden (0.03) and Canada (0.07) whereas close to the Netherlands (0.17) [7], iii) 1-OHPyr was lower than in coke-oven workers (9.7) in China [8]. Moreover, in Helsinski city, Finland, two groups (bus-garage and waste collection workers and controls) were carried out the level of exposure in summer for 2-OHNap (4.85 and 2.58), 2-OHPhe (0.09 and 0.06), 3-OHPhe (0.14 and 0.12), 1-+9-OHPhes (0.31 and 0.18) and 1-OHPyr (0.15 and 0.05). 1-OHPyr in all Thai groups was higher clearly than in Finnish (both group) while 2- and 3-OHPhes in Thai (red-taxi driver and traffic-policemen) were close to Finnish (both group) [9].

Surprisingly, the rural villagers who lived in countryside near forest where very few exposed from automobile exhausts, showed the high concentration levels of all OHPAHs, except for 1-OHNap. As possible reasons for this, the sources of PAHs and life-styles, such as open-burning for the agricultural purpose, charcoal used in their own house for cooking and warming were considered. It is interesting that the number of lung cancer incidences who live in Chiang Mai province were higher than that of other places in Thailand and other cities in Asia. Thus, the high exposure level to PAHs in Chiang Mai subjects can be related to the cause of carcinogenicity.

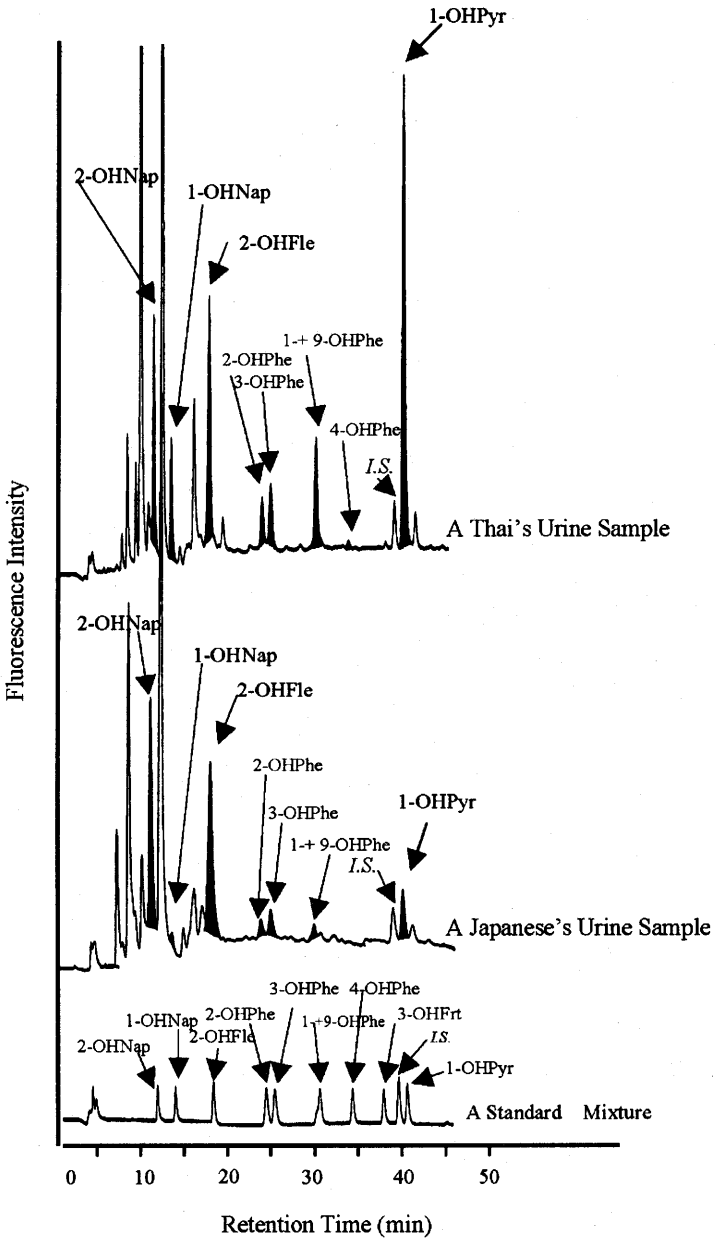

Fig. 1 HPLC chromatograms of a Thai subject, a Japanese subject and a standard mixture (10 OHPAHs)

Table 1. Urinary concentrations (μmol/mol creatinine) of ten kinds of OHPAHs in Japanese and Thai non-smokers

Non-smokers		OHPAHs metabolites								
		1-OHNap	2-OHNap	2-OHFle	1-,+9-OHPhe	2-OHPhe	3-OHPhe	4-OHPhe	3-OHFrt	1-OHPyr
Japanese	Mean ± SD	11.86 ± 12.93	0.94 ± 0.89	0.23 ± 0.24	0.09 ± 0.09	0.11 ± 0.12	0.15 ± 0.15	0.01 ± 0.0004	–	0.13 ± 0.18
(n =10)	Range	1.33 – 35.67	0.19 – 3.04	0.05 – 0.80	0.01 – 0.29	0.01 – 0.30	0.02 – 0.47	0.001 – 0.01	–	0.01 – 0.56
	No. of nd.	3	–	–	–	1	1	5	10	–
Thai: Rural villager	Mean ± SD	31.3 ± 43.8	12.13 ± 6.06	2.62 ± 1.45	0.51 ± 0.28	0.82 ± 0.41	0.78 ± 0.8	0.09 ± 0.06	–	1.2 ± 0.7
(n =10)	Range	3.3 – 141.43	2.21 – 20.16	0.63 – 5.07	0.1 – 0.84	0.12 – 1.28	0.02 – 2.46	0.02 – 0.15	–	0.17 – 2.35
	No. of nd.	1	–	–	–	–	–	–	10	–
Thai: Red-taxi driver	Mean ± SD	6.04 ± 9.09	5.32 ± 9.57	0.33 ± 0.2	0.13 ± 0.1	0.14 ± 0.11	0.17 ± 0.11	0.02 ± 0.02	–	0.26 ± 0.19
(n =10)	Range	0.68 – 16.53	0.39 – 30.51	0.09 – 0.61	0.03 – 0.34	0.02 – 0.3	0.02 – 0.42	0.01 – 0.06	–	0.06 – 0.51
	No. of nd.	6	–	–	–	–	–	2	10	–
Thai: Traffic policeman	Mean ± SD	9.22 ± 13.18	4.58 ± 6.06	0.34 ± 0.25	0.17 ± 0.12	0.16 ± 0.15	0.17 ± 0.18	0.02 ± 0.02	–	0.17 ± 0.1
(n = 10)	Range	0.04 – 37.48	0.6 – 20.74	0.11 – 0.82	0.02 – 0.32	0.05 – 0.49	0.05 – 0.6	0.002 – 0.06	–	0.05 – 0.36
	No. of nd.	3	–	–	–	–	–	–	10	–

nd; not detected

V. Conclusions

The urinary profile of OHPAHs can be served as multiple biomarkers which assess the exposure to PAHs from the environment and human activities. This result reflect that the exposure to PAHs in Thais seem to be higher than in Japanese.

Acknowledgements

This work was supported, in part, by Kanazawa university 21-Century COE Program, and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 14370728).

References

[1] T. Chetiyanukornkul, A. Toriba, R. Kizu, K. Hayakawa, "Simultaneous determination of urinary hydroxy naphthalenes, fluorene, phenanthrenes, fluoranthene and pyrene as multiple biomarkers of exposure to polycyclic aromatic hydrocarbons," submitted.
[2] T. Chetiyanukornkul, A. Toriba, R. Kizu, K. Hayakawa, "Urinary 2-hydroxyfluorene and 1-hydroxypyrene levels in smokers and non-smokers in Japan and Thailand," in press.
[3] T. Chetiyanukornkul, A. Toriba, R. Kizu, T. Makino, H. Nakazawa, K. Hayakawa, "Determination of 1-hydroxypyrene in human urine by high-performance liquid chromatography with fluorescence detection using a deuterated internal standard," *Journal of Chromatography A* Vol. 961,pp. 107-112, 2002.
[4] F.J. Jongeneelen, R.B.M Anzion, P.T.J Scheepers, R.P. Bos, P.T. Henderson, E.H. Nijenhuis, S.J. Veenstra, R.M.E. Brouns, A. Winkes, "1-Hydroxypyrene in urine as a biological indicator of exposure to polycyclic aromatic hydrocarbons in several work environments," *Annals of Occupational Hygiene* Vol.32,pp. 35-43, 1988.
[5] A. Toriba, T. Chetiyanukornkul, R. Kizu, K. Hayakawa, "Quantification of 2-hydroxyfluorene in human urine by column-switching high performance liquid chromatography with fluorescence detection," *Analyst* Vol. 128,pp. 605-610, 2003.
[6] F.J. Jongeneelen, van F.E. Leeuwen, S. Oosterink, R.B.M. Anzion, van der F. Loop, R.P. Bos, van H.G. Veen, "Ambient and biological monitoring of cokeoven workers: determinants of the internal dose of polycyclic aromatic hydrocarbons," *British Journal Industrial of Medicine*, Vol. 47,pp. 454-461, 1990.
[7] F.J. Jongeneelen, "Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons," *Annals of Occupational Hygiene* Vol. 45,pp. 3-13, 2001.
[8] M.T. Wu, C.D. Simpson, D.C. Christiani, S.S. Hecht, "Relationship of exposure to coke-oven emissions and urinary metabolites of benzo[*a*]pyrene and pyrene in coke-oven workers," *Cancer Epidemiology, Biomarkers & Prevention* Vol. 11, pp. 311-314, 2002.
[9] L. Kuusimaki, Y. Peltonen, P. Mutanen, K. Peltonen, K. Savela, "Urinary hydroxy-metabolites of naphthalene, phenanthrene and pyrene as markers of exposure to diesel exhaust," *International Archives of Occupational and Environmental Health* Vol. 77,pp. 23-30, 2004.