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SHORT COMMUNICATION

The relationship between aryl hydrocarbon hydroxylase activity and DNA adducts measured by ^{32}P-postlabelling assay in lymphocytes of lung cancer patients

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We have investigated the correlation between DNA adduct levels and aryl hydrocarbon hydroxylase (AHH) activity in peripheral lymphocyte samples obtained from 42 lung cancer patients. DNA adducts and AHH activity were determined by the ^{32}P-postlabelling technique and the fluorometric method, respectively. The mean ± SD of DNA adduct level was 0.88 ± 0.37 (ranged from 0.22 to 1.90) per 10^8 nucleotides. The geometric means of non-induced and 3-methylcholanthrene (MC)-induced AHH activity, as well as AHH inducibility (MC-induced AHH activity/non-induced AHH activity) were 0.029, 0.228 pmol min^{-1} 10^{-6} cells, and 7.776, respectively. There was no statistically significant correlation between DNA adduct levels and non-induced or MC-induced AHH activity. A tendency of positive correlation was found between DNA adduct levels and AHH inducibility for the all subjects ($n = 42$, $r = 0.25$, $p = 0.11$). Such a positive correlation reached statistical significance in the subjects with squamous cell carcinoma ($n = 13$, $r = 0.70$, $p < 0.01$). In addition, similar correlation of DNA adducts with AHH inducibility was also observed in the GSTM1 present genotype ($n = 17$, $r = 0.44$, $p = 0.07$) and GSTP1-AA genotype ($n = 29$, $r = 0.37$, $p = 0.05$) individuals. These findings suggest that DNA adduct levels are mediated by CYP1A1 enzyme, and AHH inducibility may be a more relevant indicator than specific AHH activity for explaining the variation of DNA adduct levels in lymphocytes.

Keywords: DNA adduct, aryl hydrocarbon hydroxylase, lung cancer.

Abbreviations: AHH, aryl hydrocarbon hydroxylase; CYP, cytochrome P450; GST, glutathione S-transferase.

Introduction

Lung cancer is presently the most common malignancy in the world. Epidemiological research provides that tobacco use is a major causative factor for lung cancer (IARC 1986). Polycyclic aromatic hydrocarbons (PAHs) are one of the major classes of carcinogens present in cigarette smoke. These PAH components

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are enzymatically activated and then converted into DNA-binding intermediates, namely DNA adducts (Guengerich 1988, Ioannides and Parke 1990). Such DNA adducts have been thought to be a key event in tumour initiation, and a useful biomarker for smoking-related carcinogen exposure in humans (Beach and Gupta 1992, Farmer 1995).

Most carcinogens are both activated by phase I enzymes, represented by cytochrome P450 (CYP) (e.g. CYP1A1), and detoxified by phase II enzymes, such as glutathione S-transferases (GSTs) (e.g. GSTM1, GSTP1) before binding to DNA. Aryl hydrocarbon hydroxylase (AHH) is a marker enzyme of the CYP1A1 gene. It is known to catalyse the first step in the metabolisms of benzo[*a*]pyrene (BP) and other PAH components. Some studies have associated elevated risk of lung cancer with AHH activity in cultured mitogen-stimulated lymphocytes (Emery *et al*. 1978, Lieberman 1978, Kouri *et al*. 1982, Karki *et al*. 1987).

It is possible that the activity of certain drug-metabolizing enzymes, such as AHH, should reflect the rate of activation and inactivation of carcinogens, and thus affect the formation of DNA adducts in target tissues. Two previous studies on lung cancer patients have shown a good linear correlation between pulmonary microsomal AHH activity and DNA adduct levels, measured by the ^{32}P-postlabelling method (Geneste *et al*. 1991) or high-performance liquid chromatography/fluorometric assay (Alexandrov *et al*. 1992), in non-neoplastic lung parenchyma samples.

Since blood samples are more easily obtained from the general population, we have therefore examined whether the DNA adduct levels are correlated to AHH activity using peripheral lymphocytes obtained from lung cancer patients as a surrogate tissue source for lung in the present study. Moreover, we have analysed for the first time the correlation between these two parameters in different histological types of cancer and genotypes of the GSTM1 and GSTP1.

Subjects and methods

Forty-two Japanese lung cancer patients (mean age ± SD, 64 ± 10 years), who were newly diagnosed at Kyushu University Hospital (Research Institute for Diseases of Chest, Kyushu University) from September 1995 to October 1996, participated in the present study. A questionnaire was completed for each patient to collect data regarding recent diet, illness and smoking habit. The original study population has been described in elsewhere (Kiyohara *et al*. 1999). Descriptions of the characteristic of the subjects, including sex, smoking habit, and distribution of histological type of cancer, are summarized in table 1. None of the patients were receiving drugs known to be human enzyme inducers and prior X-ray treatment or chemotherapy. All participants gave informed consent.

Venous blood was drawn from 42 patients at 9:00–10:00 a. m. and processed within 2–3 h after collection. Heparin (40 U ml^{-1}) was used as an anticoagulant. The blood sample was applied to Ficoll–Conray solution (specific gravity, 1.077) and spun at 1500 rpm at room temperature for 30 min. We then collected a lymphocyte-rich fraction that formed a white fluffy ring on top of the Ficoll–Conray solution. DNA was extracted from a small portion of the lymphocyte-rich fraction for determining DNA adducts and genotypes of GSTM1 and GSTP1.

The nuclease P1 modification of the ^{32}P-postlabelling method (Reddy and Randerath 1986, Ichiba *et al*. 1996) was used. Mainly, sample of DNA (5 µg) was hydrolysed with micrococcal nuclease and spleen phosphodiesterase and then with nuclease P1. The digest was dried and taken up in a total of 2 µl of T4 polynucleotide kinase labelling mixture containing [y-^{32}P] ATP. The labelled DNA adducts were then spotted and developed on polyethyleneimine-cellulose thin layer chromatography (TLC) plates (Macherey–Nagel, Duren, Germany) using three solvent systems: direction 1, 1 M sodium phosphate, pH 6.0; direction 3, 3.6 M lithium formate, 8.5 M urea, pH 3.5; direction 4, 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0. Adduct spots were visualized by a Bio-Image Analyzer (BAS2000, Fuji Photo Film, Tokyo, Japan) after exposing TLC plates to the Fuji imaging plate. A diagonal radioactive area on the TLC plate was counted and a background level obtained from the same plate was subtracted. Adduct levels were calculated by the labelling efficiency of the standard dAp. The results were given as a

total number of adducts per 10^8 normal nucleotides. All the analyses were repeated at least twice.

AHH activity was detected using a fluorometric method described by Kiyohara *et al*. (1990). Briefly, the lymphocyte-rich fraction was purified, washed and then incubated at 37 °C in an atmosphere of fully humidified air with 5 % CO_2. At 48 h 5 µl of 3-methylcholanthrene (MC) in acetone, to give final concentration of 2.5 µM, was added for the measurement of the MC-induced AHH activity. In a control culture, the acetone alone was added to measure the non-induced AHH activity. The incubation was then continued for an additional 48 h period. The cells from the culture flasks were harvested, washed and assayed for AHH activity at 37 °C for 50 min with BP as a substrate. The result was expressed as pmole equivalents of 3-hydroxy BP formed per min per 10^6 cells.

The GSTM1 genotype was determined by the PCR procedure described by Katoh *et al*. (1996). The presence and absence of a 215-bp amplification product resulted in two subgroups corresponding to GSTM1 present and GSTM1 null. The A-G polymorphism at codon 104 of GSTP1 gene was detected using a restriction fragment length polymorphism (RFLP)-PCR method proposed by Harries *et al*. (1997). A 176-bp PCR fragment was amplified and then digested with Alw 261. Subjects were categorized as three subgroups, wild homozygotes (AA), heterozygotes (AG) and mutant homozygotes (GG).

As the distributions of non-induced and MC-induced AHH activity were skewed to the left side, AHH activity data were log-transformed before statistical analysis. Simple linear regression was utilized to test the correlation between DNA adducts and AHH activity. Two-sided *p*-values are given.

Results and discussion

Several biomarker data measured in the present study are summarized in table 1. The results of genotyping of the GSTM1 and GSTP1 showed that 17 (40 %) individuals had the GSTM1 present genotype, and 29 (69 %) subjects had the GSTP1-AA genotype. The mean ± SD value of DNA adducts for the overall subjects was 0.88 ± 0.37 adducts per 10^8 nucleotides. There were no significant differences on DNA adduct levels when compared among subjects categorized according to sex, smoking habit, histological type of cancer, as well as genotypes of the GSTM1 and GSTP1 (data not shown). The geometric means of non-induced and MC-induced AHH activity, and AHH inducibility (MC-induced AHH activity/non-induced AHH activity) were 0.029, 0.228 pmol $min^{-1}10^{-6}$ cells, and 7.776, respectively. Large inter-individual variation was demonstrated by the 16- to 30-fold range in these AHH data.

A tendency of positive correlation of DNA adduct levels with AHH inducibility in lymphocytes was observed ($n = 42$, $r = 0.25$, $p = 0.11$). There was no significant correlation between DNA adducts and non-induced or MC-induced

Table 1. Descriptive data and several biomarkers detected in the present study in 42 lung cancer patients.

Male (number, %)	27	64 %	
Smoker (number, %)	29	69 %	
Histological type of cancer (number, %)			
Squamous cell carcinoma	13	31 %	
Adenocarcinoma	22	52 %	
Small cell carcinoma	5	12 %	
Large cell carcinoma	2	5 %	
GSTM1 present (number, %)	17	40 %	
GSTP1-AA (number, %)	29	69 %	
DNA adducts (per 10^8 nucleotides) (mean, SD, range)	0.88	0.37	0.22–1.90
Non-induced AHH activity (pmol min^{-1} 10^6 cells) (GM, GSD, range)	0.029	2.173	0.006–0.163
MC-induced AHH activity (pmol min^{-1} 10^6 cells) (GM, GSD, range)	0.227	2.092	0.048–1.476
AHH inducibility (GM, GSD, range)	7.776	2.092	1.756–32.073

GM: geometric mean, GSD: geometric standard deviation.

AHH activity. The study on the relationship between the AHH activity and DNA adducts is so far limited. Two previous studies reported a good positive correlation between DNA adducts and specific AHH activity in lung parenchyma samples (Geneste *et al*. 1991, Alexandrov *et al*. 1992). The inconsistency of outcome among different studies may be resulted from the difference of sample used, such as peripheral lymphocytes or lung tissue. On the other hand, Kiyohara and Hirohata (1997) and Kiyohara *et al.* (1998) recently confirmed that AHH activity may be influenced by age, smoking, coffee intake, and sampling season, and indicated that AHH inducibility is a much better indicator than specific AHH activity.

In addition, considering a possible modulating effect, we examined the correlation of DNA adducts with AHH inducibility in different smoking habit, histological types of cancer, and the GSTM1 and GSTP1 genotypes. As expected, a good positive correlation between DNA adducts and AHH inducibility was found among the patients with squamous cell carcinoma (figure 1, $n = 13$, $r = 0.70$, $p < 0.01$), while this correlation was relatively weak in the adenocarcinoma patients ($n = 22$, $r = 0.28$, $p = 0.20$). As sample size was small, the same analysis was not carried out in small cell carcinoma and large cell carcinoma. Squamous carcinoma has been demonstrated to more closely relate with tobacco smoking than adenocarcinoma (Barbone *et al*. 1997). Our finding provided support for this. The correlation of DNA adducts and AHH inducibility in relation to the GSTM1 and GSTP1 polymorphism is illustrated in figure 2. The DNA adduct levels were found to be significantly associated with AHH inducibility in the GSTP1-AA genotype (figure 2(B), $n = 29$, $r = 0.37$, $p = 0.05$) subjects. Similarly, a positive correlation between the two parameters was also observed in the GSTM1 present genotype patients, though it was not significant (figure 2(A), $n = 17$, $r = 0.44$, $p = 0.07$). We did not find a statistically significant correlation of DNA adducts with AHH inducibility among smokers ($n = 29$, $r = 0.25$, $p = 0.19$).

In conclusion, our results suggest that DNA adduct levels are mediated by

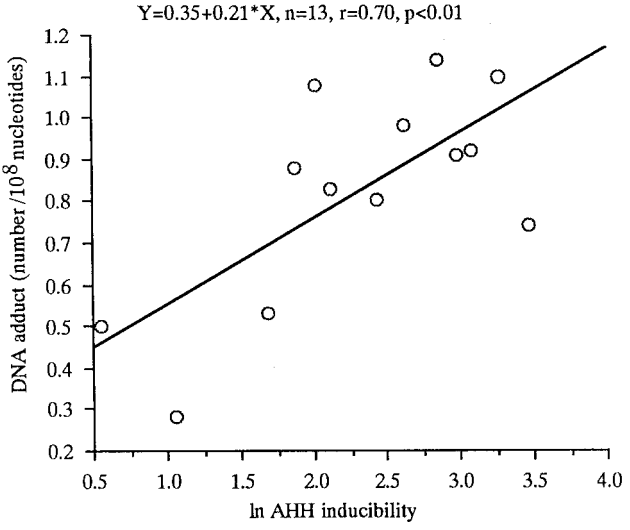

Figure 1. The correlation between DNA adduct levels and AHH inducibility in squamous cell carcinoma patients.

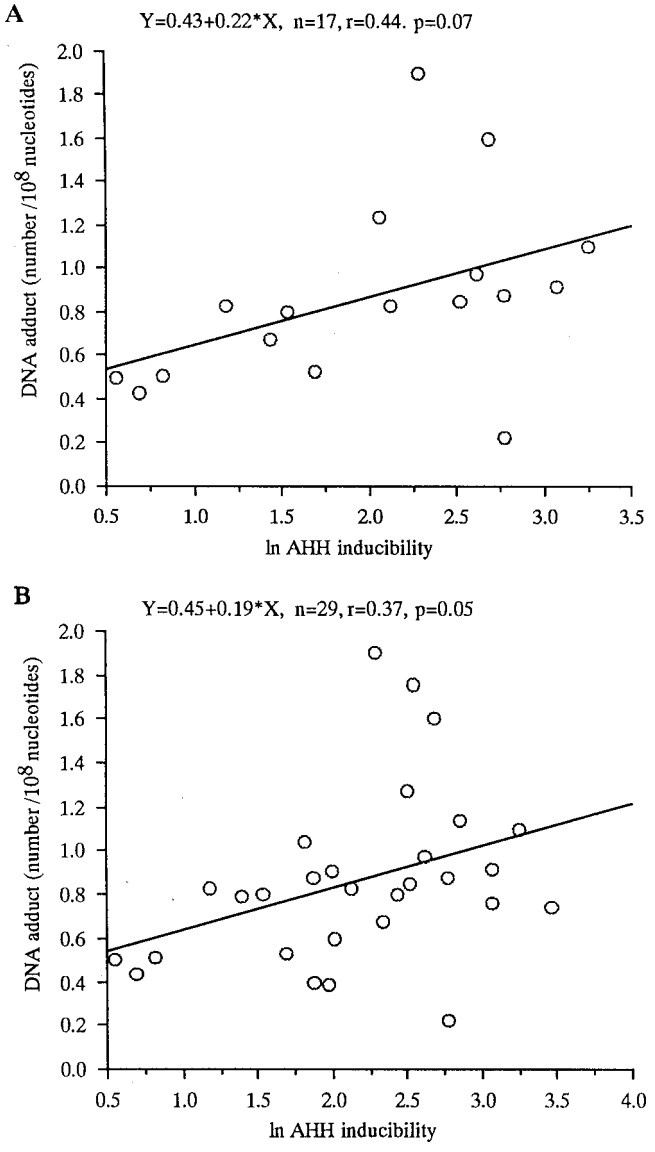

Figure 2. The correlation between DNA adduct levels and AHH inducibility in the GSTM1 present (A) and GSTP1-AA (B) patients.

CYP1A1 enzyme, and AHH inducibility may be a more relevant indicator for explaining the variation of DNA adduct levels in lymphocytes. It is also helpful for our understanding of the mechanisms of formation of DNA adducts.

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References

ALEXANDROV, K., ROJAS, M., GENESTE, O., CASTEGNARO, M., CAMUS, A. M., PETRUZZELLI, S., GIUNTINI, C. and BARTSCH, H. 1992, An improved fluorometric assay for dosimetry of benzo(*a*)pyrene diol-epoxide–DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Research*, **52**, 6248–6253.

BARBONE, F., BOVENZI, M., CAVALLIERI, F. and STANTA, G. 1997, Cigarette smoking and histologic type of lung cancer in men. *Chest*, **112**, 1474–1479.

BEACH, A. C. and GUPTA, R. C. 1992, Human biomonitoring and the ^{32}P-postlabeling assay. *Carcinogenesis*, **13**, 1053–1074.

EMERY, A. E. H., ANAND, R. and CANFORD, N. 1978, Aryl hydrocarbon-hydroxylase inducibility in patients with cancer. *Lancet*, **1**, 470–472.

FARMER, F. B. 1995, Monitoring of human exposure to carcinogens through DNA and protein adduct determination. *Toxicology Letters*, 82–83, 757–762.

GENESTE, O., CAMUS, A. M., CASTEGNARO, M., PETRUZZELLI, S., MACCHIARINI, P., ANGELETTI, C. A., GIUNTINI, C. and BARTSCH, H. 1991, Comparison of pulmonary DNA adduct levels. Measured by ^{32}P-postlabelling and aryl hydrocarbon hydroxylase activity in lung parenchyma of smokers and ex-smokers. *Carcinogenesis*, **12**, 1301–1305.

GUENGERICH, F. P. 1988, Roles of cytochrome P450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Research*, **48**, 2946–2954.

HARRIES, L. W., STUBBINS, M. J., FORMAN, D., HOWARD, G. C. W. and WOLF, C. R. 1997, Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis*, **18**, 641–644.

IARC: International Agency for Research on Cancer 1986, *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. No. 38, Tobacco Smoking* (Lyon: IARC).

ICHIBA, M., WANG, Y., OISHI, H., IYADOMI, M., SHONO, N. and TOMOKUNI, K. 1996, Smoking-related DNA adducts and genetic polymorphism for metabolic enzymes in human lymphocytes. *Biomarkers*, **1**, 211–214.

IOANNIDES, C. and PARKE, D. V. 1990, The cytochrome P450 1 gene family of microsomal hemoproteins and their role in the metabolic activation of chemicals. *Drug Metabolism Reviews*, **22**, 1–85.

KARKI, N. T., POKELA, R., NUUTINEN, L. and PELKONEN, O. 1987, Aryl hydrocarbon hydroxylase in lymphocytes and lung tissue from lung cancer patients and controls. *International Journal of Cancer*, **39**, 565–570.

KATOH, T., NAGATA, N., KURODA, Y., ITOH, H., KAWAHARA, A., KUROKI, N., OOKUMA, R. and BELL, D. A. 1996, Glutathione S-transferase M1 (GSTM1) and T1 (GST1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. *Carcinogenesis*, **17**, 1855–1859.

KIYOHARA, C. and HIROHATA, T. 1997, Environmental factors and aryl hydrocarbon hydroxylase activity (CYP1A1 phenotype) in human lymphocytes. *Journal of Epidemiology*, **7**, 244–250.

KIYOHARA, C., MOHRI, N., HIROHATA, T., HARAGUCHI, K. and MASUDA, Y. 1990, In vitro effect of methylsulfonyl polychlorinated biphenyls and 7, 8-benzoflavone on aryl hydrocarbon hydroxylase activity in human lymphoblastoid cells. *Pharmacology and Toxicology*, **66**, 273–276.

KIYOHARA, C., TANAKA, K. and HIROHATA, T. 1998, Effect of seasonal change on aryl hydrocarbon hydroxylase activity in human lymphocytes in a Japanese population. *Medical Science Research*, **26**, 75–78.

KIYOHARA, C., ICHIBA, M., ZHANG, J., NAKANISHI, Y., TAKAYAMA, K., HARA, N. and HIROHATA, T. 1999, Relationship between aromatic-DNA adduct levels and inducibility of the drug metabolizing enzyme aryl hydrocarbon hydroxylase in lymphocytes. *Medical Science Research*, **27**, 651–655.

KOURI, R. E., MCKINNEY, C. E., SLOMIANY, D. J., SNODGRASS, D. R., WRAY, N. P. and MCLEMORE, T. L. 1982, Positive correlation between high aryl hydrocarbon hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. *Cancer Research*, **42**, 5030–5037.

LIEBERMAN, J. 1978, Aryl hydrocarbon hydroxylase in bronchogenic carcinoma. *New England Journal of Medicine*, **298**, 686–691.

REDDY, M. V. and RANDERATH, K. 1986, Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. *Carcinogenesis*, **7**, 1543–1551.