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Different susceptibility to polycyclic aromatic hydrocarbons (PAH)-induced DNA damage in lung tissue in male and female non-smokers

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The levels of benzo(*a*)pyrene diol-epoxide (BPDE)–DNA adducts and polycyclic aromatic hydrocarbons (PAH) were analysed in a limited number of samples of autoptic lung tissue obtained from non-professionally exposed male ($n = 13$) and female ($n = 12$) non-smokers in an attempt to evaluate the relationship between gender, lung PAH levels ($n = 25$) and susceptibility to BPDE–DNA adduct formation ($n = 18$). Lung concentrations of chrysene, benzo(*g,h,i*)perylene and benzo(*a*)pyrene were significantly higher in males than in females ($P < 0.05$), whereas BPDE–DNA adduct levels were similar in both genders: 1.64 ± 0.42 per 10^8 bases ($\mu \pm$ SE) in males ($n = 9$) and 1.88 ± 0.64 per 10^8 bases in females ($n = 9$). Although the sample size of this study precludes any generalization, the observed data suggest that there is a potentially higher risk of PAH-induced lung DNA damage in females than in males.

Keywords: polycyclic aromatic hydrocarbons, BPDE–DNA adducts, human lung carcinogenesis.

Abbreviations: B(*a*)A, benz(*a*)anthracene; B(*a*)P, benzo(*a*)pyrene; B(*b*)F, benzo(*b*)fluoranthene; BGP, benzo(*g,h,i*)perylene; B(*k*)F, benzo(*k*)fluoranthene; BPDE, benzo(*a*)pyrene-diol-epoxide; CHRY, chrysene; DBA, dibenz(*a,h*)anthracene; PAH, polycyclic aromatic hydrocarbons.

Introduction

Several studies indicate differing individual susceptibility to carcinogens such as PAH and aromatic amines in humans (Shields *et al.* 1993). Large interindividual variations have been documented in the activity of enzymes involved in the biotransformation of carcinogens such as aryl hydrocarbon hydroxylase (Alexandrov *et al.* 1992), acetyltransferases (Vineis *et al.* 1990) and glutathiontransferases (Tang *et al.* 1998) and a difference in metabolic rate related to gender has also been reported (Mollerup *et al.* 1999). It has therefore been suggested that the ability to activate/detoxify carcinogens or DNA repair (Rudiger *et al.* 1989) may affect an individual's risk for lung cancer (Lang and Kadlubar 1992).

Polynuclear aromatic hydrocarbons (PAH) are ubiquitous in the environment and their carcinogenic effects are well documented (IARC 1986). The main sources of human PAH exposure are polluted air, tobacco smoke and contaminated food (Binkova *et al.* 1995, Lodovici *et al.* 1995). As a measure of the internal dose,

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PAH–DNA adducts have been measured in target tissue such as the lung (Alexandrov *et al.* 1992, Shields *et al.* 1993) and in other more accessible surrogate tissues such as white blood cells (Rojas *et al.* 1995, Lodovici *et al.* 1999).

We previously published data on benzo(*a*)pyrene diol-epoxide (BPDE)–DNA adducts in autoptic lung tissues, demonstrating a higher level of BPDE–DNA in smokers and we also observed that PAH levels were lower in a limited number of samples from female non-smokers (data not reported) (Lodovici *et al.* 1998). As a follow-up to this later observation we attempted to examine the levels of BPDE–DNA adduct in autoptic lung tissue samples in non-smoking males and females with no professional exposure in an increased number of samples.

Materials and methods

Study population

Human lung specimens ($n = 25$) were obtained from autopsies conducted by the Department of Pathology, University of Florence for routine microscopy. Following autopsy they were immediately frozen at –20 °C in the Department of Pathology until analysis, which was completed within 30 days. All samples were obtained from the same area of the lungs (apex of left inferior lobe). Three lung samples were conserved, divided into three pieces to be analysed weekly in order to assess the relative stability of PAH and BPDE–DNA adduct levels under our experimental conditions. After a period of 7 or 14 days at room temperature no significant reduction was observed in the levels of BPDE–DNA adducts and PAH levels (data not shown). We analysed samples from deceased subjects who were non-smokers (subjects who never smoked) who had resided in the Florence area (central Italy), with no occupational PAH exposure. This information was obtained from the clinical records and verified through personal interviews with the families. A source of PAH exposure is through contaminated food, but we do not have information of differential PAH exposure relative to gender in Italy.

All individuals analysed had died of various causes other than lung cancer. In 18 autoptic lung samples, nine from females and nine from males (as reported in Lodovici *et al.* 1998) we measured PAH and BPDE–DNA adduct levels. PAH levels were also measured in seven additional autoptic lung samples (four females, three males).

Extraction and detection of PAHs

The following PAHs: benz(*a*)anthracene (B(*a*)A), benzo(*b*)fluoranthene (B(*b*)F), benzo(*k*)fluoranthene (B(*k*)F), chrysene (CHRY), benzo(*a*)pyrene (B(*a*)P), dibenz(*a,h*)anthracene (DBA) and benzo(*g,h,i*)perylene (BGP) were determined in human lung autoptic tissues using our published method (Lodovici *et al.* 1998).

BPDE–DNA adduct analysis

Adducts were isolated as previously reported (Lodovici *et al.* 1998). Briefly, protein and RNA were removed from the tissue by extensive digestion with proteinase K and RNAse and by solvent extraction. After purification DNA was hydrolysed and, in order to remove fluorescent impurities from B(*a*)P-tetrols, the hydrolysed DNA solution was passed through a C18 Sep-Pak cartridge (Waters, Milan, Italy). Non-tetrol material was removed with H_2O and 20% methanol and B(*a*)P-tetrols were eluted with 55% methanol. Recovery of tetrols from the Sep-Pak column was about 80%. The eluates were evaporated and analysed by HPLC, as previously reported; data were expressed as the sum of *syn* and *anti*-BPDE–DNA adduct levels (Lodovici *et al.* 1998). In the preliminary part of the experimental set-up four samples were processed thrice in order to evaluate the reproducibility of our experimental design. The values obtained from the ratio of *anti* or *syn* BPDE–DNA adducts over total base content in three repeated sets of samples showed no noticeable difference. Consequently, only samples that yielded 0 or higher than 4×10^8 bases we processed in duplicate. The average value of two determinations are reported where the samples ($n = 8$) were processed in duplicate.

Results and discussion

Few data have been published on background PAH levels in lung tissue of non-smokers (Tomingas *et al.* 1976, Seto *et al.* 1993) and even fewer are available on

Table 1. Levels of individual PAH (ng g^{-1} dry weight, μ± SE) in autoptic lung samples.

	n	Age range	BGP	B(*a*)A	CHRY	B(*b*)F	B(*k*)F	B(*a*)P	DBA[a]	PAH[a]
Males	12	61–85	0.61±0.08	0.44±0.14	9.67±2.7	0.41±0.1	0.197±0.01	0.48±0.09	0.058±0.015	11.86±0.89
Females	13	70–84	0.37±0.05	0.31±0.05	2.23±0.55	0.37±0.06	0.168±0.01	0.20±0.03	0.048±0.002	3.7±0.19
Significance (*P*)			0.024	–	0.026	–	–	0.049	–	0.018

[a] PAH : total PAH levels (sum of 7).

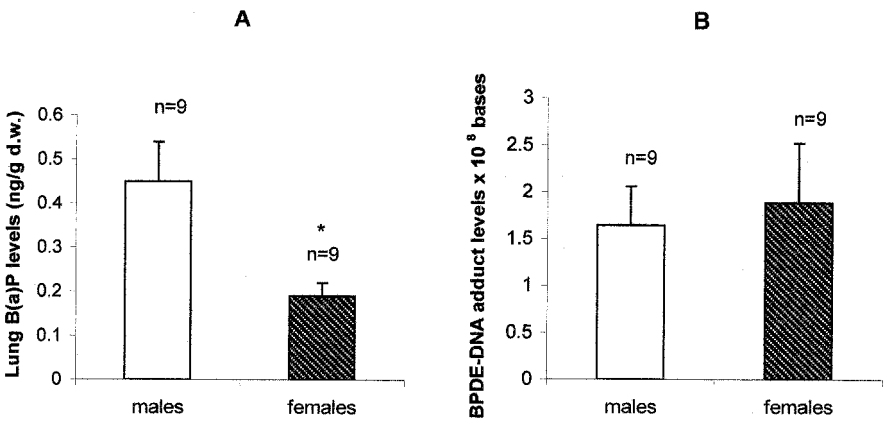

Figure 1. B(*a*)P levels (Panel A) and BPDE–DNA adduct levels (Panel B) in autoptic lung samples from male and female non-smokers in which both determination were performed. The values are expressed as means ± SE. * $P < 0.05$.

human lung PAH–DNA adduct levels (Ryberg *et al*. 1994). In the present study we measured PAH and BPDE–DNA adduct levels in autoptic lung samples from male and female non-smokers in order to evaluate the relationship between lung PAH levels, susceptibility to BPDE–DNA adduct formation and gender. Table 1 reports individual PAH levels found in the lung tissues obtained from male and female non-smokers.

These data show that lung tissues obtained from female non-smokers have lower total PAH levels ($P = 0.018$), particularly BGP, CHRY and B(*a*)P, compared with males. Similarly, Seto *et al*. (1993) previously reported that three PAH in the lung samples from non-smokers were significantly higher in males than in females and explained this difference by suggesting that females have higher levels of PAH-metabolizing enzymes. However, no data are reported in the literature regarding PAH-metabolizing activity in the lungs of male and female non-smokers; whereas Mollerup *et al*. (1999) found that female smokers had a significantly higher expression of lung CYP1A1 compared with males.

In our study, notwithstanding the fact that B(*a*)P concentrations in lungs from males were significantly higher ($P = 0.049$) than those from females (table 1 and figure 1, panel A), BPDE–DNA adduct levels detected in lung tissues from male and female non-smokers were similar, as shown in figure 1, panel B. This similarity persisted even in data derived from samples obtained from rural dwellers with respect to those from urban areas (data not shown).

Our observations need to be confirmed with a larger sample size. However, the data suggest a higher susceptibility of women to PAH-induced lung DNA damage.

No correlation was found between lung adducts ($r = -0.340$, $n = 18$), PAH levels ($r = 0.166$, $n = 25$) and age of subjects.

These data agree with the observation of Ryberg *et al.* (1994) who reported similar PAH–DNA adduct levels in normal tissue from male and female with lung cancer non-smokers. In the same study it was also reported that after adjusting for smoking exposure, DNA adduct levels in females were higher than in males. More recently, Mollerup *et al.* (1999) reported that non-cancerous lung tissue from smoking females had a significantly higher level of aromatic/hydrophobic DNA adducts compared with males. This observation was explained, at least in part, by a gender-related difference in metabolic activation, particularly by CYP1A1 expression levels, whereas these authors found similar DNA adduct levels in non-smoking males and females, in agreement with our results.

However, besides CYP1A1, other enzymes such as glutathione S-transferase or DNA repair, could contribute to sex differences in lung DNA damage from PAH. Given the age range of the deceased subjects (61–84) and their prevailing habits for that generation, men and women have a markedly different life-style (men often spending time in recreational facilities polluted by cigarette smoke); in contrast the exposure through food is probably similar with respect to gender.

In conclusion, our data, although preliminary, provide a further possible mechanism of a difference in susceptibility to PAH between men and women in lung carcinogenesis. A higher susceptibility of females to tobacco lung carcinogenesis had been described before and was explained on the basis of a differential expression of a specific gene related to bronchial cell proliferation (Shriver *et al.* 2000).

Our data also imply that background PAH exposure is capable of inducing measurable DNA damage in non-smokers.

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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 fluorimetric assay for dosimetry of benzo(*a*)pyrene diol-epoxide–DNA adducts in smokers' lung: comparison with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Research*, **52**, 6248–6253.

Binkova, B., Lewtas, J., Miskova, I., Lenicek, J. and Sram, M. 1995, DNA adducts and personal air monitoring of carcinogenic polycyclic aromatic hydrocarbons in an environmentally exposed population. *Carcinogenesis*, **16**, 1037–1046.

IARC 1986, *Tobacco Smoking*. Monograph No. 38 (Lyon: International Agency for Research on Cancer), pp. 7–81.

Lang, N. P. and Kadlubar, F. F. 1992, The human glutathione S-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. *Environmental Health Perspectives*, **98**, 87–94.

Lodovici, M., Dolara, P., Casalini, C., Ciappellano, S. and Testolin, G. 1995, Polycyclic aromatic hydrocarbon contamination in the Italian diet. *Food Additives and Contaminants*, **12**, 703–713.

Lodovici, M., Akpan, V., Giovannini, L., Migliani, F. and Dolara, P. 1998, Benzo(*a*)pyrene diol–epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. *Chemico-Biological Interactions*, **116**, 199–212.

Lodovici, M., Akpan, V., Casalini, C., Nencini, L., Pinzauti, M., Marcoccia, M. and Dolara, P. 1999, Benzo(*a*)pyrene diol-epoxide–DNA adduct levels and arylhydrocarbon hydroxylase (AHH) in human white blood cells from smokers and non-smokers. *Biomarkers*, **4**, 272–280.

MOLLERUP, S., RYBERG, D., HEWER, A., PHILLIPS, D. H. and HAUGEN, A. 1999, Sex differences in lung CYP1A1 expression and DNA adduct levels among lung cancer patients. *Cancer Research*, **59**, 3317–3320.

ROJAS, M., ALEXANDROV, K, AUBURTIN, G., WASTIAUX-DENAMUR, A., MAYER, L., MAHIEU, B., SEBASTIAN, P. and BARTSCH, H. 1995, Anti-benzo(*a*)pyrene diol-epoxide–DNA adduct levels in peripheral mononuclear cells from coke oven workers and the enhancing effect of smoking. *Carcinogenesis*, **16**, 1373–1376.

RUDIGER, H. W., SCHWARTZ, U., SERRAND, E., STIEF, M., KRAUSE, T., NOWAK, D., DOERJER, G. and LEHNERT, G. 1989, Reduced O^6 methylguanine repair in fibroblast cultures from patients with lung cancer. *Cancer Research*, **49**, 5623–5626.

RYBERG, D., HEWER, A., PHILLIPS, D. H. and HAUGEN, A. 1994, Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. *Cancer Research*, **54**, 5801–5803.

SETO, H., OHKUBO, T., KANOH, T., KOIKE, M., NAKAMURA, K. and KAWAHARA, Y. 1993, Determination of polycyclic aromatic hydrocarbons in the lung. *Archives of Environmental Contamination and Toxicology*, **24**, 498–503.

SHIELDS, P. G., BOWMAN, E. D., HARRINGTON, A. M., DOAN, V. T. and WESTON, A. 1993, Polycyclic aromatic–DNA adducts in human lung and cancer susceptibility genes. *Cancer Research*, **53**, 3486–3492.

SHRIVER, S. P., BOURDEAU, H. A., GUBISH, C. T., TIRPAK, D. L., LUKETICH, J. D. and SIEGFRIED, J. M. 2000, Sex-specific expression of gastrin-releasing peptide receptor: relationship to smoking history and risk of lung cancer. *Journal of the National Cancer Institute*, **92**, 24–33.

TANG, D. L., RUNDLE, A., WARBURTON, D., SANTELLA, R. M., TSAI, W. Y., CHIAMPRASERT , S., HSU, Y. Z. and PERERA, F. R. 1998, Associations between both genetic and environmental biomarkers and lung cancer : evidence of a greater risk of lung cancer in women smokers. *Carcinogenesis*, **19**, 1949–1953.

TOMINGAS, R., POTT, F. and DEHNEN, W. 1976, Polycyclic aromatic hydrocarbons in human bronchial carcinoma. *Cancer Letters*, **1**, 189–196.

VINEIS, P., CAPORASO, N., TANNEMBAUM, S., SKIPPER, P., GLOGOWSKI, J., BARTSCH, H., CODA, M., TALASKA, G. and KADLUBAR, F. 1990, The acetylation phenotype, carcinogen–hemoglobin adducts, and cigarette smoking. *Cancer Research*, **50**, 3002–3004.