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Benzo(a)pyrene diolepoxide–haemoglobin and albumin adducts at low levels of benzo(a)pyrene exposure

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A biomonitoring study was conducted to simultaneously measure individual benzo(*a*)pyrene (BaP) exposure in 50 office employees, not occupationally exposed to polycyclic aromatic hydrocarbons (PAH), using personal samplers and the formation of (+) *r*-7, *t*-8-dihyroxy-*t*-9,*t*-10-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (BPDE) adducts to haemoglobin (BPDE–Hb) and serum albumin (BPDE–SA). The population enrolled was exposed to an average of 0.58 ± 0.46 ng BaP m^{-3} (mean ± SD). The concentration of BaP collected from smokers' samples was double that from non-smokers ($P = 0.007$). BPDE adducts to Hb and SA were quantified as BaP tetrols released from hydrolysis of macromolecules and measured by high-resolution gas chromatography–negative ion chemical ionization–mass spectrometry. BPDE–Hb adducts were detected in 16% of the population and BPDE–SA adducts in 28%. Smoking did not affect adduct formation. When BaP personal monitoring data were used as the criterion of exposure, no correlation was found with the presence and the levels of BPDE–Hb and BPDE–SA adducts. Undetected sources of PAH, such as the diet, might markedly alter the exposure profile depicted by individual air sampling and affect the frequency and levels of protein biomarkers. This is the first comparative analysis of BPDE–Hb and BPDE–SA adducts, providing reference values for these biomarkers in a general urban population. However it is difficult to establish which biomarkers would be the more relevant in assessing low BaP exposure, due to undetectable factors such as dietary PAHs, that might have influenced the results to some degree.

Keywords: benzo(*a*)pyrene diolepoxide adducts, haemoglobin, albumin, urban population.

Abbreviations: BaP, benzo(*a*)pyrene; PAH, polycyclic aromartic hydrocarbons; BPDE, (+) *r*-7, *t*-8-dihyroxy-*t*-9,*t*-10-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene; Hb, haemoglobin; SA, serum albumin; BPT, benzo(*a*)pyrene tetrols; PEM, personal exposure monitoring.

Introduction

One of the major classes of carcinogenic agents in a polluted atmosphere is polycyclic aromatic hydrocarbons (PAH), which have been associated with increased risk of neoplastic diseases (IARC 1983).

The best known PAH is benzo(*a*)pyrene (BaP), often used as a model compound of PAH exposure. BaP is metabolically activated to (+) *r*-7, *t*-8-

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dihyroxy-*t*-9,*t*-10-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (BPDE), the ultimate carcinogenic metabolite able to bind covalently to DNA and blood proteins in humans (Gelboin *et al* 1980, Naylor *et al*. 1990).

Because genotoxic compounds can react with haemoglobin (Hb) and serum albumin (SA) to form stable adducts, assessment of PAH–blood protein adducts has been considered an interesting alternative to dosimetry based solely on measured PAH ambient levels and PAH metabolites in body fluids. Since the lifetime of Hb in humans is about 120 days and the half-life of SA is approximately 25 days, and no enzymatic repair system exists for these proteins, measurement of blood protein adducts might reflect the integrated exposure to PAH in the 1–4 months before sampling. In addition, protein adduct-based dosimetry may reflect individual differences in metabolism (Tannenbaum *et al*. 1993).

BPDE adducts to Hb and SA have been used to assess BaP exposure mostly in occupational cohorts with high levels of exposure (Ferreira *et al*. 1994, Omland *et al*. 1994, Tas *et al*. 1994) and so far there is limited information about the usefulness of these biomarkers in the general population. The majority of comparative analyses of BaP blood protein adducts, however, have been done in animal models, reporting higher levels of BPDE adducts to SA (BPDE–SA) than to Hb (BPDE–Hb) (Viau *et al*. 1993, Viau and Carrier 1995).

We reported earlier that the presence of BPDE–Hb may be related to traffic exhaust among newspaper vendors in the city of Milan, Italy (Pastorelli *et al*. 1996). In this investigation we examined a group of office employees living in Milan, as a model urban population exposed to low levels of PAH, to test which BPDE blood protein adduct is most useful for assessing BaP exposure. We also investigated the correlation between BPDE–Hb and BPDE–SA adducts and BaP levels based on personal monitoring.

Materials and methods

Subjects and sampling

Fifty people were analysed in this investigation, part of a population enrolled in a previous study (11). Briefly, they were clerks, 25 men and 25 women, aged 18–60 years (mean ± SD 37 ± 10), living and working in the urban area of Milan. Thirty-seven were non-smokers and 13 were moderate smokers.

A standardized questionnaire was administered to each subject to record age, current smoking habits, passive smoking exposure, medical treatments and diet, including grilled or smoked food. Personal exposure to respirable particulate matter was monitored for 24 h and measurements were made during the working day.

Informed consent was obtained and a blood sample was collected when the environmental monitoring was completed.

Recruitment, air and biological sampling were done, as previously described (Pastorelli *et al*. 1999a), by members of the Department of Occupational and Environmental Health, University of Milan, during February–March 1996.

Methods

Details of the methods used for BaP personal exposure monitoring (PEM) and BaP air analysis have been previously reported (Minoia *et al*. 1997, Pastorelli *et al*. 1999a). Briefly, a single air measurement was performed per subject. A personal sampler operated continuously for a 24-h period and was fitted on the individuals near the breathing zone and kept near their bed overnight. BaP in environmental particulate samples was analysed using high pressure liquid chromatography coupled with a fluorescence detector. The assay detection limit was = 0.02 ng BaP m^{-3}.

BPDE protein adducts were analysed as benzo(*a*)pyrene tetrols (BPT) released from proteins and quantitated by high-resolution gas chromatography–negative ion chemical ionization–mass spectrometry with selected ion recording (HRGC-NICI-SIR) as previously reported (Pastorelli *et al*. 1996, 1998). The detection limit of the method was 0.05 fmol BPT mg^{-1} protein.

Statistical analysis

Correlations were checked by the Spearman rank-order correlation test. To compute mean adduct levels, persons with unmeasurable levels were considered as having half the minimum detectable value. The Mann–Whitney two-tailed *U*-test was used to compare adduct levels in different groups of exposure. A difference was considered significant at $p<0.05$.

Results

The average exposure to BaP detected by 24-h personal monitoring in office employees was 0.58 ± 0.46 ng m^{-3} (mean ± SD), with a range of = 0.02–1.81 ng BPT m^{-3}. It should be noted however that only a single air measurement was made. Table 1 reports the exposure to BaP in all individuals, in relation to their smoking habits. Smokers had double the levels of non-smokers. The difference was statistically significant ($P = 0.007$).

BPDE–Hb adducts reached a mean (± SD) of 0.14 ± 0.38 fmol BPT mg^{-1} Hb as reported in detail elsewhere (Pastorelli *et al.* 1999a). BPDE–SA addducts ranged from = 0.05 to 0.54 fmol BPT mg^{-1} with an average of 0.07 ± 0.12 fmol BPT mg^{-1} SA. BPDE–Hb and BPDE–SA adducts were detectable in eight (16%) and 14 (28%) workers, respectively. Restricting the analysis to individuals with measurable adducts, BPDE–Hb and BPDE–SA levels were respectively 0.94 ± 0.72 fmol BPT mg^{-1} Hb (mean ± SD) and 0.19 ± 0.18 fmol BPT mg^{-1} SA. No association was found between the presence of BPDE–Hb and BPDE–SA adducts.

Hb and SA adducts in smokers were 0.12 ± 0.36 fmol BPT mg^{-1} Hb and 0.09 ± 0.14 fmol BPT mg^{-1} SA. These values were no different from those in non-smokers, where adducts to Hb were 0.14 ± 0.39 fmol BPT mg^{-1} Hb and adducts to SA were 0.06 ± 0.11 fmol BPTmg^{-1} SA. No other life-style factors (alcohol, xanthines and grilled food intake) correlated with BPDE adduct levels.

Figure 1 illustrates the percentage of detectable adducts in subjects grouped into BaP exposure categories. Individuals exposed to more than 0.6 ng BaP m^{-3} did not show any significant difference in BPDE–Hb or BPDE–SA detectable adducts (11.8% and 23.5% respectively) compared with individuals exposed to less than 0.3 ng BaP m^{-3} (16.7% and 33.3%). Levels of BPDE–Hb and BPDE–SA adducts were not significantly different in relation to BaP exposure classes, although people in the lowest exposure group showed a tendency to higher levels of adducts than those in the highest exposure group (table 2).

Table 1. Levels of BaP detected by 24-h personal monitoring in 50 office employees, according to their smoking habits (number of subjects analysed are in parentheses).

	BaP ng/m^{-3}	
	Non-smokers (37)	Smokers (13)
Mean ± SD	0.47 ± 0.36	0.9 ± 0.58a
Range	0.02 – 1.44	0.3 – 1.81
Median	0.36	0.6

[a] Smokers/non-smokers: $p = 0.007$, Mann–Whitney two tailed *U*-test.

Table 2. BPDE-Hb and BPDE-SA adducts levels in 50 office employees, classified according to their levels of exposure to BaP (numbers of subjects analyzed are in parentheses).

Exposure categories BaP (ng m^{-3})	Employees	BPDE-Hb (fmol BPT mg^{-1}) mean ± SD	BPDE-SA (fmol BPT mg^{-1}) mean ± SD
< 0.3	All	0.21 ± 0.54 (18)	0.08 ± 0.14 (18)
	With detectable adducts	1.13 ± 0.97 (3)	0.19 ± 0.21 (6)
0.3–0.6	All	0.09 ± 0.16 (15)	0.06 ± 0.13 (15)
	With detectable adducts	0.30 ± 0.21 (3)	0.22 ± 0.27 (3)
> 0.6	All	0.11 ± 0.31 (17)	0.05 ± 0.06 (17)
	With detectable adducts	0.75 ± 0.82 (2)	0.14 ± 0.08 (4)

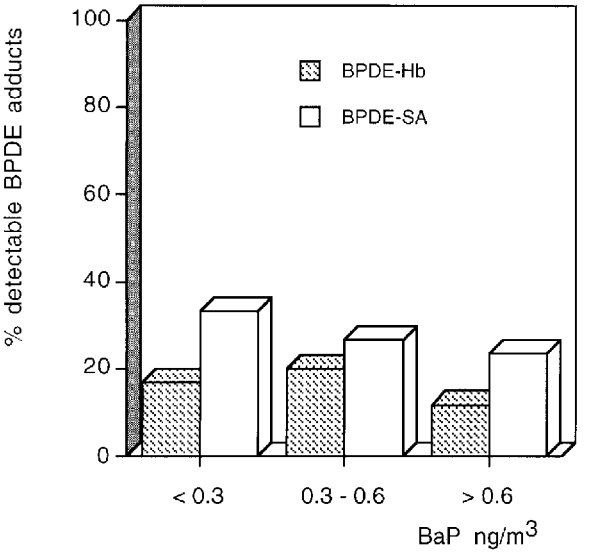

Figure 1. Detectable BPDE protein adducts in 50 office employees classified according to their levels of exposure to BaP.

Discussion

This study is part of an ongoing comprehensive evaluation of biological markers in an urban population with low levels of exposure to PAH. Of interest was the relationship between BaP exposure (based on personal exposure) and levels of BPDE adducts to Hb and SA. So far the association between PAH exposure and PAH biological markers has been shown only in occupationally-exposed workers, where airborne levels of BaP were in the range of 10–3000 ng BaP m^{-3} (Santella *et al*. 1993, Ferreira *et al*. 1994, Omland *et al*. 1994, Tas *et al*. 1994). More recently we have reported significant differences in BPDE–Hb concentrations in newpaper vendors in relation to traffic exhaust (Pastorelli *et al*. 1996), but individual exposure was not monitored in that study.

The PEM data in the present study clearly show that the workers were exposed

to levels of BaP below 1ng BaP m^{-3} comparable to those experienced by most people in other Italian cities (Cecinato *et al.* 1993, Crebelli *et al.* 1995, Merlo *et al.* 1998), but that PEM BaP significantly increased with cigarette smoking exposure.

As we already reported in heavy smokers (Pastorelli *et al.* 1998), a larger proportion of people had detectable BPDE–SA adducts than BPDE–Hb adducts, perhaps because SA is synthesized in the liver, in which most xenobiotic metabolism takes place. Furthermore, adduct formation might be expected to be more efficient with SA because the protein is not isolated by any cell membrane, as Hb in the erythrocytes, that hampers electrophile diffusion. Differences in relative reactivity with SA and Hb and differences between the assays may also play a role. We are therefore led to suggest that BPDE–SA adducts could be a more informative marker of BaP internal dose than BPDE–Hb adducts.

Smoking increases exposure to BaP and data concerning the effect of cigarette smoke on protein adducts are scanty and conflicting (Tas *et al.* 1994, Pastorelli *et al.* 1996). Our previous study (Pastorelli *et al.* 1998) found that BPDE–SA adduct levels were approximately double in heavy smokers (average of 30 cigarettes day^{-1}) compared with the moderate smokers in this study (average of 15 cigarettes day^{-1}). An average of 15 cigarettes day^{-1} yields 300–600 ng BaP inhaled per day. These figures are far higher that the estimated average atmospheric BaP inhaled daily by the clerks (11.14 ng BaP day^{-1}, assuming a breathing rate of 0.8 $m^3 h^{-1}$), but there was no significant effect on BPDE protein adducts.

The presence and levels of BPDE–Hb and BPDE–SA adducts were not correlated with BaP levels of exposure from individual samplers. However it should be noted that these adduct levels reflect exposure over several weeks or more, whereas only one exposure measurement was made. Given the small sample size and the high SD, the tendency to higher adduct levels in the lowest exposure group may have been due to chance rather than to differences in response to exposure (induction of detoxification, and/or further activation pathways) as reported for PAH high doses (Lewtas *et al.* 1997).

The lack of association between BPDE proteins adducts and BaP exposure might be due to the fact that dosimetry by personal samplers usually measures the quantity of BaP inhaled the day before sampling, while both the biological markers we analysed give indications of cumulative dose over a period of 4 months in the case of Hb or 4 weeks for SA. This result does not exclude that BPDE protein adducts can differentiate between exposure levels. We found that BPDE–SA adducts levels were 2.2 times significantly higher in occupationally-exposed workers (0.14 fmol BPT mg^{-1} SA $\pm$ 0.22, mean $\pm$ SD) than their reference controls (0.06 fmol BPT mg^{-1} SA $\pm$ 0.1, mean $\pm$ SD; $P < 0.01$), that showed average BPDE–SA amount similar to that measured in the employees of the present study (Pastorelli *et al.* 1999b). Albeit we previously reported BPDE–Hb adducts to be enhanced in winter (Pastorelli *et al.* 1999a), the average BPDE–Hb level in clercks, sampled in winter was approximately 3.2 times lower than in newspaper vendors sampled in summer (Pastorelli *et al.* 1996). It is reasonable to assume that the long daily outdoor exposure to air pollution experienced by the newspaper vendors in Milan, Italy increases BPDE–Hb levels.

When assessing the value of PAH exposure biomarkers to indicate the true dose absorbed by an individual, the diet has to be taken into consideration as a potentially confounding factor, since cooking methods and food processing may contribute to the amount of PAH ingested by people (Lioy *et al.* 1988, Waldman *et al.* 1991).

Unfortunately, it proved difficult to estimate the real BaP dietary exposure by a questionnaire alone, as we did, since PAHs are present in a wide variety of foods (Lodovici *et al*. 1995, Philips 1999). In this study alcohol intake had no apparent effect. Eating grilled or smoked food was not correlated with BPDE protein adducts, presumably because it is not widespread habit in Italy. It has been estimated that the daily intake of BaP in the Italian diet is about 200–300 ng, essentially from eggs, dairy products and cereals (Lodovici *et al*. 1995, E. Zuccato personal communication), but this can easily vary depending on the foods consumed. These figures are 20–30 times higher than the average BaP inhaled by the enrolled employees (11.14 ng BaP day^{-1}) and closer to the estimated amount inhaled by a moderate smoker (300 ng BaP day^{-1}). Therefore the exposure profile estimated by individual air sampling could be markedly altered by ingested PAH. As suggested by animal studies (van de Wield *et al*. 1993, Gerde *et al*. 1997), the route of exposure may govern not only BaP bioavailability, but also which metabolic pathways are most important in BaP activation and detoxification, leading to the modulation of adduct formation.

In conclusion, this is the first simultaneous analysis of BPDE blood protein adducts in the general population. Although BPDE–SA adducts are more frequently detected than BPDE–Hb adducts, it is difficult to establish which biomarker would be the most suitable in assessing BaP exposure. At low environmental BaP exposure, it seems likely that additional factors which are currently not precisely measurable, such as dietary PAH, may also be important in adducts formation.

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