Interindividual variation in the levels of certain urinary polycyclic aromatic hydrocarbon metabolites following medicinal exposure to coal tar ointment

Elise D. Bowman, Nathaniel Rothman, Christian Hackl, Regina M. Santella and Ainsley Weston

Determination of human exposure to polycyclic aromatic hydrocarbons (PAHs) is challenging because they are so broadly distributed in the environment and often difficult to quantitate using questionnaire methods. To enhance the ability to non-invasively evaluate markers of both internal dose and biologically effective dose we have developed methods for the identification and quantitation of 1-hydroxypyrene–glucuronide and *r*-7,*t*-8,*t*-9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BP-7,10/8,9-tetrol) in human urine. In the current study we applied these assays to urine samples collected from 43 hospitalized psoriasis patients treated with coal tar medication and 39 non-treated volunteer controls. BP-7,10/8,9-tetrol was detected in 20 of 43 (47%) patients, ranging from ≤1 (not detected) to 124 fmol μmol^{-1} creatinine. In contrast, BP-7,10/8,9-tetrol was detected in only 4 of 39 (10%) controls, range ≤1 to 20.6 fmol μmol^{-1} creatinine (p = 0.0006, Wilcoxon rank sum test). A second, more polar PAH metabolite, identified as 1-hydroxypyrene–glucuronide, was present in all urine samples. Mean 1-hydroxypyrene–glucuronide levels were 40.96±72.62 pmol μmol^{-1} creatinine in patients and 0.38±0.32 pmol μmol^{-1} creatinine in control subjects (p ≤0.0001). The ratio of urinary levels of BP-7,10/8,9-tetrol to 1-hydroxypyrene–glucuronide was examined in the coal tar-treated patients. This ratio was found to vary by approximately 6000-fold. This parameter cannot be explained by measurement error because the coefficients of variation for these assays are only 12 and 10% respectively, nor can it be explained by use of different coal tar products. These results provide further evidence that substantial interindividual variation in activation of benzo[*a*]pyrene and other PAHs exists, which may have implications for disease risk.

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Abbreviations: *BP, benzo*[a]*pyrene; BPDE, r-7,t-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; BP-7,10/8,9-tetrol, r-7,t-8,t-9,c-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene; ELISA, enzyme linked immunosorbant assay; GC/MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; ND, not detected; PAH, polycylcic aromatic hydrocarbon; PC, personal computer; SD, standard deviation; UVB, ultraviolet B.*

Introduction

Case reports and epidemiological observations between the 18th and 21st centuries indicate an association between exposure to certain complex chemical mixtures (soot, cutting oils and coal tar) and skin cancer in humans (Philips 1983). Medicinal application of coal tar to psoriasis patients presents the opportunity to study the metabolic fate of carcinogens in humans. The current study examines the metabolism of a potent carcinogen, BP, and a co-carcinogen, pyrene (Van Duuren and Goldschmidt 1976, Rice *et al.* 1984).

Extensive environmental analyses have shown that BP and other PAHs contaminate air, food, soil, and water, making human exposure unavoidable (IARC 1973). Biomarkers of exposure provide valuable tools to assess the extent and significance of human contact. A number of methods for monitoring human exposure to environmental or occupational carcinogens have been developed. These include: measurements of mutagens in urine, determination of PAH–DNA adducts in human tissues by ELISA assays, ^{32}P-postlabelling, fluorescence or mass spectrometry, measurement of aromatic adducts in surrogate macromolecules and measurement of 1-hydroxypyrene in urine (Ames *et al.* 1975, Jongeneelen *et al.* 1985, Poirier and Weston 1996).

The development of assays that use urine for biomarker detection is important for human biomonitoring because sample collection is simple, non-invasive and the amounts of material available for study are relatively large. Urinary assays were initially used to indirectly determine the presence of mutagens and carcinogens using the Ames mutagenicity tests (Ames *et al.* 1975). More sophisticated and chemically specific assays have previously been used to detect PAH metabolites such as 1-hydroxypyrene and 3-hydroxybenzo[*a*]pyrene by fluorescence detection after HPLC (Jongeneelen *et al.* 1987, Arise *et al.* 1994), as well as metabolites of PAHs by ELISA (Santella *et al.* 1994). A recent addition to these methods described the development of an assay based on immunoaffinity purification and synchronous fluorescence spectroscopy to measure BP-7,10/8,9-tetrol in human urine samples (Weston *et al.* 1993). This metabolite may be more relevant for estimating PAH associated risk for subsequent disease than measures of 1-hydroxypyrene or 3-hydroxybenzopyrene because activ

formation of BPDE (an ultimate carcinogen) must occur prior to formation of the corresponding tetrahydrotetrol.

High PAH exposures are encountered through smoking tobacco (IARC 1986) and the use of medicinal coal tar preparations for skin conditions, such as psoriasis, as part of the Goeckerman therapy (Goeckerman 1925). Therapeutic coal tar ointment is formed from products of the pyrolytic decomposition of coal under thermodynamic conditions, and coal tar contains a wide range of substances. In addition to an assortment of PAHs, coal tar contains a large number of compounds with organically bound sulphur, nitrogen, and oxygen, mostly in the form of heterocyclic compounds (Wright *et al.* 1985). The mechanism of the therapeutic action of this distillation product has not been clearly described, but it has been demonstrated that PAHs are absorbed through the skin (Storer *et al.* 1984).

Here we report on the detection of PAH metabolites (specifically BP-7,10/8,9-tetrol and the glucuronide conjugate of 1-hydroxypyrene) in psoriasis patients treated therapeutically with coal tar ointment. Urine samples were analysed from 43 treated patients and 39 untreated, volunteer controls. The BP-7,10/8,9-tetrol and glucuronide conjugate of 1-hydroxypyrene were extracted from the urine using silica columns, immunoaffinity chromatography and HPLC. Metabolites were detected by synchronous fluorescence spectroscopy, and tested for associated smoking history, diet and demographic characteristics of study subjects.

MATERIALS AND METHODS

Subject enrolment

Patients were recruited from the in-patient service of the Department of Dermatology at the Presbyterian Hospital at Columbia-Presbyterian Medical Center. Subjects were enrolled and consent obtained using Institutional Review Board-approved procedures. Eligibility criteria for cases were a diagnosis of plaque-stage psoriasis and having undergone modified Goeckerman therapy for at least 1 day (typically 7–14 days). Controls were recruited by advertisement in the medical centre, and individually-matched to patients by age (2-year intervals), sex and current tobacco smoking status. Exclusion criteria for controls were use of coal tar preparations (e.g. shampoos) or psoriasis. Initially, 57 patients and 53 controls were recruited as previously reported (Santella *et al.* 1994). Using different methods to those described here, Santella *et al.*, (1994) reported on a battery of PAH tests for a larger set of psoriasis patient samples that include the subset reported here. Their method for measurement of urinary 1-hydroxypyrene was based on that of Jongeneelen *et al.* (1985), and although measurements for coal tar-treated persons were comparable to those reported here, many control levels were below the limit of detection, which precludes comparison. Goals of the current study were to use a more sensitive and specific assay for 1-hydroxypyrene–glucuronide levels and additionally measure the benzo[*a*]pyrene-7,10/8,9-tetrol. For the current study, urine samples (24 h) were available for analysis of only 43 cases and 39 controls; statistical calculations were limited to 37 controls because of the lack of creatinine levels for two of the control subjects.

A questionnaire was administered by trained interviewers that requested demographic information, current occupation, tobacco smoking history and a brief history of recent consumption of foods with relatively high levels of PAHs. For cases, medical records were abstracted for the number of days each subject had been treated with coal tar ointments and for current medications.

Chemicals

Affinica Antibody Orientation Kit Protein A Agarose was obtained from Schleicher and Schuell (Keene, NH). β-glucuronidase (type-B3) was purchased from Sigma Chemical Corp. (St Louis, MO). (±)-*r*-7,*t*-8-Dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydro[1,3-^{3}H]-benzo[*a*]pyrene was purchased from Chemsyn Scientific Laboratories (Lenexa, KA) through the National Cancer Institute Chemical Carcinogen Reference Standard Repository (Bethesda, MD) and hydrolysed to the BP-7,10/8,9-tetrol in aqueous acid (0.1 N HCl, 90 °C, 3 h). Solid phase extraction cartridges (C18 Sep-Pak Plus) were purchased from Millipore Corp. (Milford, MA) and C18 reverse phase HPLC columns were purchased from Vydac (Hesperia, CA). All other reagents were HPLC grade or comparable (J. T. Baker, Phillipsberg, NJ). GC/MS analysis of coal tar ointment was performed by META Environ, Inc. (Boston, MA).

Antibody and immunoaffinity columns

An anti-N_2-(7,8,9-trihydroxy[10-yl])guanosin-7,8,9,10-tetrahydrobenzo[*a*]pyrene monoclonal antibody was purified from a hybridoma cell line (8E11) (Santella *et al.* 1984). This antibody, which is known to have a broad substrate specificity (Bowman *et al.* 1990), was coupled to the Protein A Agarose following the manufacturer's directions and packed into plastic columns with a total binding capacity of > 200 nmol BP-7,10/8,9-tetrol ligand. Preparation of anti-BP-7,10/8,9-tetrol immonoaffinity columns is described in detail elsewhere (Weston *et al.* 1993).

Sample preparation

Urine samples were prepared as previously described (Weston *et al.* 1993). Briefly, urine samples (10 ml) were acidified to 0.1 N HCl with concentrated acid and heated at 90 °C for 3 h to release conjugated BP metabolites. Reverse phase solid phase extraction cartridges (C-18) were prepared by pre-washing with methanol (6 ml) and then flushing with water (12 ml). The cooled urine samples (4 °C) were loaded onto the cartridges and then washed with water (6 ml). Hydrophobic compounds were eluted with methanol (6 ml, 80%), dried *in vacuo* to remove the methanol, and resuspended in Tris–HCl (6 ml, 10 mM, pH 7.5). This eluate was loaded onto the 8E11 immunoaffinity columns (anti-PAH) and washed with Tris–HCl (6 ml, 10 mM, pH 7.5). Bound material was eluted with methanol in water (60%, 2 ml) and the volume was reduced under reduced pressure to 500 μl. Urinary creatinine levels were determined using a kit from Sigma Chemical Co. (St Louis, MO).

High performance liquid chromatography and detection of PAH-metabolites

Liquid chromatography was performed using a Gilson HPLC system. A Vydac C-18 reverse phase column was eluted with a linear gradient (30 to 60% methanol over 20 min and a 100% methanol wash between samples). HPLC fractions were dried under reduced pressure to remove solvent and resuspended in water (500 μl) and analysed using a Perkin Elmer MPF-66 fluorescence spectrophotometer (Norwalk, CT) in the synchronous scanning mode.

For BP-7,10/8,9-tetrol appropriate fractions (18.5–19.0 min) were analysed. BP-7,10/8,9-tetrol has a peak emission at 379 nm when the monochromators are driven synchronously with a wavelength difference of 34 nm. Samples were quantitated by comparison of fluorescence yields (peak heights) with a standard-curve determined using dilutions of authentic BP-7,10/8,9-tetrol. The limit of detection for this assay is 2.5 fmol ml^{-1} for a 10 ml urine sample.

To address the question of reproducibility, six aliquots (10 ml) from a person testing negative for BP-7,10/8,9-tetrol were dispensed from a single fresh urine sample, and known quantities of tritiated BP-7,10/8,9-tetrol were added to three of them. All six samples were handled in a manner

obtained from the psoriasis patients and their controls. After HPLC, the samples were analysed for BP-7,10/8,9-tetrol by both synchronous fluorescence spectroscopy and liquid scintillation counting of appropriate fractions. The coefficient of variation for this assay was determined to be 12% (Weston *et al.* 1994, Kang *et al.* 1995).

For 1-hydroxypyrene–glucuronide conjugate elution (6 min), samples were also subjected to fluorescence analysis using the same conditions. Determination of the absolute levels was made from comparison with the BP-7,10/8,9-tetrol standard curve using a 4.5× correction factor since the fluorescence quantum yield for the 1-hydroxypyrene–glucuronide metabolite is 4.5× less than that for BP-7,10/8,9-tetrol (Bowman *et al.* 1990, Strickland *et al.* 1994). The detection limit for this metabolite is 12 fmol ml^{-1} urine, given a 10 ml urine sample (Bowman *et al.* 1993, Weston *et al.* 1993, 1994). The coefficient of variation for this assay has been determined to be 8–10% (Bowman *et al.* 1993, Weston *et al.* 1994, Kang *et al.* 1995). To confirm the identity of the 1-hydroxypyrene–glucuronide these fractions were digested (37 °C, 30 min) by mixing β-glucuronidase (300 U) with sodium acetate buffer (100 mM, pH 5.0) and an equal volume of the substrate (aqueous HPLC fraction). Materials were then subjected to HPLC (Strickland *et al.* 1994). For the purpose of determining BP-7,10/8,9-tetrol/1-hydroxypyrene–glucuronide (×100) ratios, samples in which BP-7,10/8,9-tetrol could not be detected were assigned a value of half the limit of detection.

Statistical analysis

Data were analysed using SAS® for PC version 6.04 (SAS® Institute, Inc. Cary, NC, 1987). Summary values are expressed as mean (standard deviation) and as median (range). Since only subsamples of the original patients and controls were available for analysis (based upon availability of urine samples), unmatched tests of significance were used. However, the original matching variables (i.e. age, sex, smoking status) were evaluated for potential confounding for each outcome. Group differences in each variable were tested by the Wilcoxon rank sum test for continuous variables, and by Fisher's exact test for categorical variables. Spearman rank order correlation was used to test for the association between each biomarker and various sources of PAHs (i.e. diet, tobacco smoking and the number of treatment days for patients).

The distribution of 1-hydroxypyrene–glucuronide levels was normalized with a natural logarithmic transformation. Since the BP-7,10/8,9-tetrol distribution could not be normalized with standard transformations, a rank transformation was used to allow for multivariable analyses (Conover and Iman 1981). Group differences in each biomarker were then adjusted for age, race, sex, and current tobacco smoking status (yes/no; and number of cigarettes smoked per day) using analysis of covariance. Only the range of values for metabolites in these samples were previously documented in a report describing the methodology (Weston *et al.* 1994). The current report focuses on interindividual variation in metabolite levels.

Results

Concentrations of selected PAHs in a representative coal tar ointment sample are presented in Table 1. These measurements were consistent with previously published data (IARC 1985). The data obtained here indicated that five-fold more pyrene (700 mg kg^{-1}) than benzo[*a*]pyrene (140 mg kg^{-1}) was present in these preparations. Table 2 shows demographic data for the psoriasis patients (n = 43) and controls (n = 39). No significant difference between the two groups was found for any of these variables (age, gender, race or cigarette smoking) using Fisher's exact test.

Although there were more African American controls than patients, and more Latino patients than controls, the overall racial distribution was not significantly different between the two groups (p = 0.18). Only three (7%) controls and four (10%) patients reported consumption of any charbroiled meat within the previous 2 weeks. Of these, only two controls and no patients had eaten charbroiled meat within the last 48 h. Since PAH metabolites from PAHs in the diet are excreted mostly within 24 h (Buckley and Lioy 1992,

Phenanthrene	1600
2-Methylnaphthalene	1200
Fluoranthene	860
Acenaphthene	840
Fluorene	770
Pyrene	700
Dibenzofuran	620
1-Methylnaphthalene	510
Anthracene	460
Naphthalene	430
Chrysene	250
Benz[*a*]anthracene	220
Benzo[*b*]fluoranthene	160
Benzo[*a*]pyrene	140
Indeno[1,2,3-*cd*]pyrene	90
Benz[*g,h,i*]perylene	80
Benzo[*k*]fluoranthene	50
Acenapthylene	ND
Dibenz[*a,h*]anthracene	ND
Indan	ND

Table 1. GC/MS analysis of medicinal coal-tar ointment. Determination of levels of polycyclic aromatic hydrocarbons (mean of three analyses, mg/kg).

Characteristics		Untreated controls, *n* (%)	Treated patients, *n* (%)
Age distribution (years):			
< 30		6 (15)	6 (14)
30–49		20 (52)	17 (40)
50–69		7 (18)	15 (34)
≥70		6 (15)	5 (12)
Gender:	Male	21 (54)	22 (51)
	Female	18 (46)	21 (49)
Race:	Caucasian	21 (54)	25 (58)
	African American	10 (25)	4 (9)
	Latino	5 (13)	11 (26)
	Other	3 (8)	3 (7)
Current smoking status:			
	Non-smoker	27 (69)	30 (70)
	Smoker	12 (31)	13 (30)
Consumption of charbroiled meat[a]			
	None	40 (93)	35 (90)
	1 or more	3 (7)	4 (10)

Table 2. Demographic characteristic of case control study subjects.

[a] Number of portions in last week.

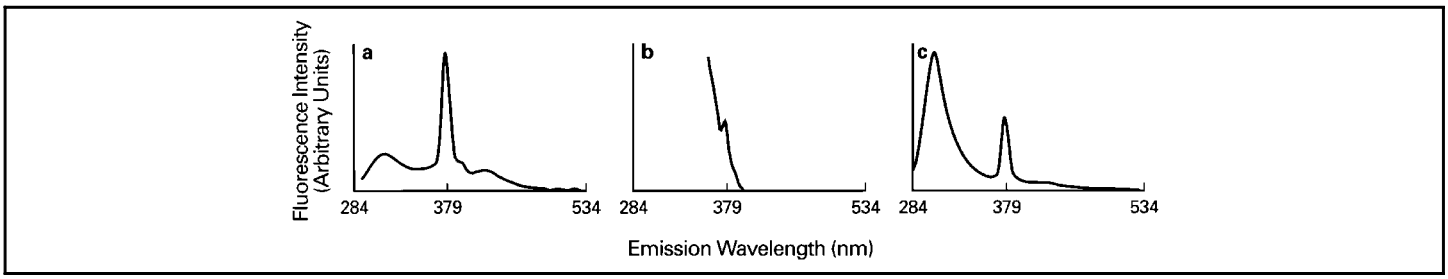

Figure 1. Representative synchronous fluorescence spectra ($\Delta\lambda$ 34 nm) for materials isolated from the urine of a coal tar-treated psoriasis patient. (a) Results of immunoaffinity purification only using an anti-N_2-(7,8,9-trihydroxy[10-yl])guanosin-7,8,9,10-tetrahydrobenzo[*a*]pyrene monoclonal antibody with affinity to other PAH metabolites; (b) further purification of materials from the immunoaffinity eluates using HPLC, spectrum for materials with an HPLC retention time equal to that of BP-7,10/8,9-tetrol (18.5–19.0 min); (c) further purification of materials from the immunoaffinity eluates using HPLC, spectrum for materials with an HPLC retention time equal to that of 1-hydroxypyrene–glucuronide (5.5–6.0 min).

association between this variable and measured PAH metabolites was not assessed in subsequent analyses.

Extracts of urine containing tritium-labelled BP-7,10/8,9-tetrol were analysed by HPLC to determine the retention time and recovery (Weston *et al.* 1993, 1994). The retention time was determined to be between 18.5 and 19 min with 32–34% recovery. Figure 1 is a composite of three synchronous fluorescence spectra ($\Delta\lambda$ 34 nm), each generated separately for materials isolated from human urine. Figure 1(a) shows a representative spectrum for materials isolated from the urine of a psoriasis patient following immunoaffinity chromatography but prior to HPLC. These materials have a spectrum which is characteristic of the pyrene fluorophore, however, the materials are a mixture of compounds captured on the affinity columns by the anti-BPDE antibodies. Figure 1(b) shows a fluorescence spectrum of material isolated from the same sample (Figure 1a) by HPLC that had a retention time identical to that of BP-7,10/8,9-tetrol. BP-7,10/8,9-tetrol was detected in the urine of 20 of 43 (47%) coal tar-treated psoriasis patients and 4 of 39 (10%) controls (Table 3). Levels of BP-7,10/8,9-tetrol determined in urine were significantly higher in psoriasis patients (not detected to 124 fmol μmol^{-1} creatinine) than in untreated controls (not detected to 20.6 fmol μmol^{-1} creatinine) (p = 0.0006, Table 3).

A more polar metabolite was also detected by synchronous fluorescence spectroscopy after HPLC in these samples (retention time 6 min) (Figure 1c). This compound has almost identical spectral characteristics to BP-7,10/8,9-tetrol by synchronous fluorescence spectroscopy and by comparison of fluorescence excitation–emission matrices (data not shown) (Bowman *et al.* 1993, Weston *et al.* 1993). The metabolite was present in all samples, controls and patients alike. Further chemical characterization has shown this compound to be the glucuronic acid conjugate of 1-hydroxypyrene (Strickland *et al.* 1994); this was confirmed using β-glucuronidase digestion and HPLC.

The 1-hydroxypyrene–glucuronide conjugate was found in all study subjects; however, higher levels were found in coal tar treated cases than untreated controls. The mean 1-hydroxypyrene–glucuronide levels were 40.96±72.62 (range 0.54–463.90 pmol μmol^{-1} creatinine) for patients and 0.38±0.32 (range 0.04–1.32 pmol μmol^{-1} creatinine) for controls. The levels of the 1-hydroxypyrene–glucuronide conjugate were significantly greater in the coal tar-treated patients than controls ($p \leq 0.0001$, Table 3). Group differences for each biomarker were minimally affected after adjustment for age, sex, race and current tobacco smoking. None of these variables themselves were associated with either biomarker, in either patients or controls (data not shown). Among patients, total number of treatment days was correlated with the 1-hydroxypyrene–glucuronide conjugate (r = 0.19, p = 0.23, n = 41) and BP-7,10/8,9-tetrahydrotetrol (r = 0.30, p = 0.05, n = 41) but was only statistically significant for the latter.

PAH metabolite	Untreated controls (n = 37)[a]	Treated patients (n = 43)	p value for difference[b]
Levels of BP-7,10/8,9-tetrol[c]			
Mean (±SD)	1.3 (4.2)	15.0 (29.5)	
Median (range)	0 (0–20.6)	0 (0–124.0)	p = 0.0006
Levels of 1-hydroxypyrene[d]			
Mean (±SD)	0.38 (0.32)	40.96 (72.62)	
Median (range)	0.30 (0.04–1.32)	22.69 (0.54–463.90)	$p \leq 0.0001$

Table 3. Urinary polycyclic aromatic hydrocarbon metabolites in coal tar-treated psoriasis patients and controls.

[a] Creatinine levels were unavailable for two control individuals.

[b] Wilcoxon rank sum test.

[c] fmol μmol^{-1} creatinine, 20 (47%) of patients and 4 (10%) of controls had levels above the detection limit of the assay.

[d] pmol μmol^{-1} creatinine, all study subjects had detectable levels.

Figure 2 shows the relationship between BP-7,10/8,9-tetrol and 1-hydroxypyrene–glucuronide levels for each individual in the study. Among patients, BP-7,10/8,9-tetrol and 1-hydroxypyrene–glucuronide levels were moderately correlated (Spearman r = 0.31, p = 0.05). For the entire study group, Figure 2 shows that there was substantial variation in the tendency to form BP-7,10/8,9-tetrol over a wide range of 1-hydroxypyrene–glucuronide; some patients with very high levels of 1-hydroxypyrene–glucuronide still had undetectable levels of BP-7,10/8,9-tetrol

In order to further explore interindividual variation in excretion of these two metabolites, the ratio of urinary BP-7,10/8,9-tetrol to 1-hydroxypyrene–g

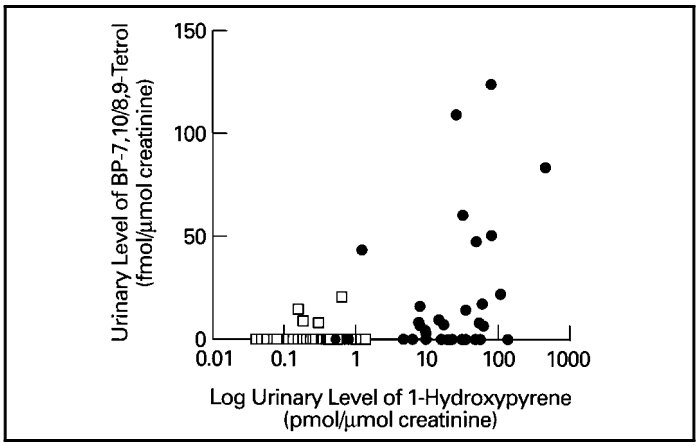

Figure 2. Correlation of urinary BP-7,10/8,9-tetrol levels (fmol μmol^{-1} creatinine) with those of 1-hydroxypyrene–glucuronide (pmol μmol^{-1} creatinine) in coal tar-treated psoriasis patients and untreated volunteer controls.

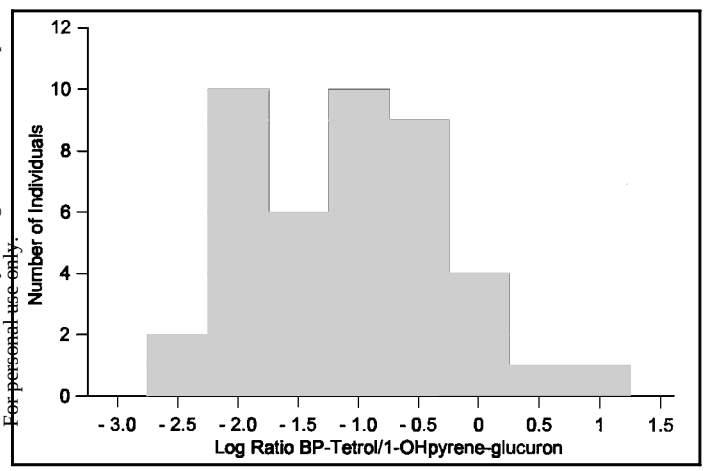

Figure 3. Histogram of the distribution of polycyclic aromatic hydrocarbon metabolite ratios (benzo[*a*]pyrene-7,10/8,9-tetrahydrotetrol [BP-tetrol]/ 1-hydroxypyrene–glucuronide [1-OHpyrene-glucuron]) among 43 coal tar-treated psoriasis patients. The bar represents the number of individuals within the range depicted on the *X*-axis. The ratio ranged from 0.0027% to 16.24%, 6015-fold.

calculated for patients (subjects that were below the level of detection for BP-7,10/8,9-tetrol were assigned a value halfway between zero and the detection limit of the assay). This ratio ranged from 0.0027% to 16.24%, more than 6000-fold (Figure 3). When the data for 23 individuals with non-detectable levels of BP-7,10/8,9-tetrol were excluded, the range remained in excess of 350-fold. When variation in this ratio was evaluated within relatively homogeneous categories of 1-hydroxypyrene–glucuronide levels (i.e. 1–10 pmol, 10–100 pmol and > 100 pmol 1-hydroxypyrene–glucuronide μmol^{-1} creatinine) there was approximately 300-fold variation in urinary BP-7,10/8,9-tetrol:1-hydroxypyrene–glucuronide within each group.

When the data were re-examined without controlling for creatinine concentration in the urine there were only minimal differences in the analyses. Levels of both BP-7,10/8,9-tetrol (coal tar treated psoriasis patients ND-330 fmol ml^{-1} and controls ND-40 fmol ml^{-1}) and 1-hydroxypyrene–glucuronide (coal tar-treated psoriasis patients 0.8–76.1 pmol ml^{-1} and controls 0.2–2.9 pmol ml^{-1}) were significantly elevated in the patients.

Discussion

The immunoaffinity/HPLC/synchronous fluorescence spectroscopy assay described for the detection of metabolites of PAHs in human urine samples is a non-invasive technique that can be used for human biomonitoring. BP-7,10/8,9-tetrol was detected in the urine of more coal tar-treated patients (20/43) than controls (4/39) and at higher levels (mean 15.0±29.5 fmol μmol^{-1} creatinine for cases and 1.3±4.2 fmol μmol^{-1} creatinine for controls, $p = 0.0006$). In addition, a glucuronide conjugate of 1-hydroxypyrene was also detected. This metabolite, stable to acid treatment (0.1N HCl, 90 °C, 3 h), was detected in all samples (controls: 40–1320 fmol μmol^{-1} creatinine and psoriasis patients: 540–463900 fmol μmol^{-1} creatinine); no samples were below the limit of detection. Thus, although a wide variation in total levels was observed, virtually no overlap was seen between exposed psoriasis patients and controls for the 1-hydroxypyrene–glucuronide ($p \leq 0.0001$, for difference). In light of the difficulties with dose assessment in coal tar-treated psoriasis patients (exposure assessment extrapolated from medical records), these methods could be of potential clinical value.

Determination of exact chemical dose in medicinal coal tar-treated patients is problematic. Patients use their hands to daub the coal tar on affected regions of skin, a nurse, using gloved hands, assists in this process to coat areas of skin inaccessible to the patient. Thus, while absolute dosage is variable from patient to patient and despite possible use of different coal tar ointments, the ratio of pyrene to benzo[*a*]pyrene should remain approximately constant at 3.5–5:1 (IARC 1985). However, the ratio of BP-7,10/8,9-tetrol:1-hydroxypyrene–glucuronide varied over a scale of more than 3 logarithms (range > 6000-fold, although we acknowledge that minimally this range could be as little as 3000-fold if all the BP-7,10/8,9-tetrol non-detectable samples were just below the detection limit of the assay). These observations show wide interindividual variation in the human population with respect to metabolism of these PAH compounds. This observation is consistent with descriptions of polymorphisms in an increasing number of enzymes that metabolize xenobiotics (Guengerich 1992, Shields *et al.* 1993, Shou *et al.* 1994), and variation among different individuals to absorb and excrete chemicals.

Two types of assay have been used to measure 1-hydroxypyrene in urine for a variety of exposure settings (occupational, tobacco, diet and medicinal). Jongeneelen *et al.* (1985, 1987, 1988) have pioneered and established a widely used assay for the detection of free 1-hydroxypyrene following reverse metabolism (β-glucuronidase/arylsulphatase) of urine extracts. Alternatively, the method used here is more sensitive because the glucuronide conjugate is approximately five-times more fluorescent than 1-hydroxypyrene (Strickland *et al.* 1994).

Jongeneelen *et al.* (1985) reported 1-hydroxypyrene levels between 0.05 (their stated detection

μmol^{-1} creatinine for controls, with no difference between smokers and non-smokers. Hansen *et al.* (1993) measured 1-hydroxypyrene in occupationally exposed individuals using an HPLC/fluorescence assay with a detection limit of 1.06 pmol ml^{-1}. The upper limit of their measured values was 1.15 pmol ml^{-1} for controls (28/32 samples were negative in the assay, i.e. ≤1.06 pmol ml^{-1} for occupational controls (with 60/122 negative) and 39 pmol ml^{-1} for occupationally-exposed individuals (6/108 negative). Schaller *et al.* (1993) measured 1-hydroxypyrene levels in smoking and non-smoking workers at a garbage incineration plant; although the range of values was similar (0.05–0.41 pmol μmol^{-1} creatinine in non-smokers and 0.07–0.41 pmol μmol^{-1} creatinine in smokers), there was a statistically significant difference between the groups (detection limit 0.5 pmol ml^{-1} using a 10 ml urine sample). In each of these studies, the levels of 1-hydroxypyrene recovered from the urine are clearly in the same range as those reported here for the 1-hydroxypyrene–glucuronide conjugate. However, it is also important to recognize that the current method is more sensitive and for all samples levels of 1-hydroxypyrene–glucuronide measured were greater than the detection limit of the assay. Most recently, Sithisarankul *et al.* (1997) reported higher levels of 1-hydroxypyrene–glucuronide in urine of smokers (1.04 pmol ml^{-1}) than non-smokers (0.55 pmol ml^{-1}).

Kang *et al.* (1995) have measured levels of 1-hydroxypyrene–glucuronide in persons before and after eating charbroiled meat. Baseline urinary levels of 1-hydroxypyrene–glucuronide were 0.23±0.11 pmol ml^{-1} which rose to 6.50±1.50 pmol ml^{-1} during the charbroiled meat feeding period. The range of values that they observed (2.00–16.60 pmol ml^{-1}) indicated considerable interindividual variation in response to exposure to the same amount of ingested PAHs, similar observations have been made by van Maanen *et al.* (1994) and Sithisarankul *et al.* (1997).

Jongeneelen *et al.* (1985) measured 1-hydroxypyrene in a limited number of psoriasis patients treated with coal tar (n = 2). Levels of 1-hydroxypyrene were 5 and 250 pmol μmol^{-1} creatinine (extracted from 25 ml urine), values similar to those reported here.

No association was found between excretion of either BP-7,10/8,9-tetrol or 1-hydroxypyrene–glucuronide and current smoking in either patients or controls. However, there was only a relatively small number of smokers in each group (n = 12/39 for controls and 13/43 for patients); and cigarettes smoked per day was low to moderate (20±14 cigarettes/day, both groups). Thus there was limited statistical power to evaluate this effect.

Animal studies in neonatal rodents have shown that topical administration of crude coal tar preparations and ultraviolet irradiation leads to the induction of microsomal cytochrome P450 dependent enzymes such as aryl hydrocarbon hydroxylase, 7-ethoxycoumarin *O*-deethylase, epoxide hydrolase, and glutathione transferase (Das *et al.* 1985, Mukhtar *et al.* 1986) which in turn have been linked to squamous cell carcinoma. There have been several epidemiological studies and case reports that indicate that Goeckerman therapy may also lead to an increased risk of skin cancer (Stern *et al.* 1980, Bickers *et al.* 1981, Bridges *et al.* 1981, IARC 1985, Moy *et al.* 1986) in humans. However, negative studies also exist (Pittelkow *et al.* 1981, Torinuki and Tagami 1988). Additional studies are needed to resolve this question. Chemical identification of BP-7,10/8,9-tetrol, a metabolite of the potent carcinogen BPDE in human urine after exposure to coal tar, suggests that systemic circulation of PAHs occurs in patients who undergo Goeckerman therapy, and that these patients may be at increased risk of cancers at sites other than the skin, e.g. the urinary bladder (Tola 1980). The data presented here demonstrate wide interindividual variation in the metabolism of BP and urinary excretion of BP-7,10/8,9-tetrol, this is consistent with *in vitro* studies (Harris 1987). These data further suggest that some individuals may be at greater risk of cancer following medicinal exposure to coal tar products than others. Use of the biomonitoring methods described here in epidemiological studies of cancer risk in coal tar-treated psoriasis patients may help to resolve these questions.

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