Biological monitoring of human exposure to coal tar

Urinary excretion of total polycyclic aromatic hydrocarbons, 1-hydroxypyrene and mutagens in psoriatic patients

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Summary. Three methods for the biological monitoring of human exposure to coal tar were compared. Levels of 1-hydroxypyrene (1-OH PYR), polycyclic aromatic hydrocarbons (PAH) and mutagens (Ames plate incorporation assay using Salmonella typhimurium strain TA98 in the presence of 9β and 9β-glucuronidase) were determined in urinary samples from psoriatic patients undergoing topical treatment with mineral coal tar. A single sample of urine with a high content of PAH was diluted with urine of non-exposed, non-smoking subjects in order to obtain nine samples with a decreasing content of PAH metabolites. Mutagenicity of the extracts was detectable down to the dilution corresponding to a content in 1-OH PYR of about 50 μg/g creatinine and total PAH of 7 μg/g creatinine. In a second phase the three indicators of exposure to PAH were compared in 16 urinary samples from four psoriatic patients. The total PAH levels determined by the acidic deconjugation/reduction method were confirmed to be nearly always lower than the corresponding levels of 1-OH PYR alone. Most of the extracts were mutagenic, however, some of the samples with a high content in PAH metabolites were not mutagenic. In all the urinary samples analyzed the excretion of 1-OH PYR was markedly greater than in control subjects. 1-OH PYR and urinary mutagenicity levels were well correlated. The present data suggest that both the determination of mutagenicity and 1-OH PYR in urine may be used to monitor occupational exposure to PAH, the latter method being cheaper and of greater specificity and sensitivity.

Key words: Ames test – 1-Hydroxypyrene – Psoriasis – Polycyclic aromatic hydrocarbons – Urine

Introduction

Coal tar and derived products, which contain high amounts of polycyclic aromatic hydrocarbons (PAH), are classified as compounds with sufficient evidence for cancer induction in man. Several occupational exposures to coal tar pitch volatiles have been shown to be at risk for tumors of the skin and/or of the lung and in some cases of the bladder [14]. Nonetheless occupational exposure to coal tar and derived products is still widespread: approximately 145,000 workers were exposed in 1977 in the USA [27].

The diffusion of exposure to PAH and the type of risk involved justifies the interest in the development of methods for biological monitoring, three types of which have so far been proposed, based on the analysis of different urinary parameters. One method consists of the determination of the mutagenic activity of urinary extracts in the Salmonella/microsome assay. Increased urinary mutagenicity has been found in subjects treated cutaneously with therapeutical coal tar [6, 35], in coke plant workers [8, 23] and in anode plant workers [13], whereas other studies have reported negative results following occupational exposure to PAH [3, 5, 26, 31].

Another interesting method, consisting of the chemical reduction of PAH urinary metabolites to the unmetabolized form and the subsequent detection of the individual PAH by high performance liquid chromatography (HPLC) was proposed by
Becher and Bjorseth [1]. With this method, increased levels of urinary PAH were detected in anode plant workers [2, 33], while no variation was observed in coke plant workers [10]. Following cutaneous treatment with coal tar, psoriatic patients showed a very marked increase of urinary PAH metabolites detectable by this method [6].

The most recent method consists of the determination of a single urinary metabolite of pyrene, 1-hydroxy pyrene (1-OH PYR). Increased urinary levels of 1-OH PYR have been detected in patients cutaneously treated with coal tar, in workers exposed to the wood preservant creosote oil [16], in coal tar distillery workers [17] and in road paving workers using tar blended binders [20].

In the present study the above three methods are compared in order to establish the relationship between the different parameters measured as well as the sensitivity and specificity of the individual methods. The exposure to PAH was monitored in psoriatic patients treated with therapeutical coal tar preparations.

Materials and methods

**Urinary samples**

In order to determine the sensitivity of the three methods, a single sample of urine was collected from a male, non-smoking psoriatic patient, following a 3-d therapeutical exposure to pure coal tar. The sample was serially diluted (dilutions ranging from 1:2 to 1:256), using a pool of urine from non-smoking subjects not exposed to PAH. Thus a series of eight artificial urinary samples with decreasing content of PAH metabolites was obtained.

The correlation of the three methods was then determined by the analysis of a further 16 samples of urine collected from four male, non-smoking psoriatic patients, undergoing treatment with the Goeckerman regimen (cutaneous application of coal tar based ointment, followed by exposure to UV irradiation) in the Dermatology Clinic of the University of Padua. One patient (A) was treated with pure coal tar (1 d), the other three (B, C, D) were treated with 4% coal tar based ointment respectively for 2, 8 and 13 d. Body surface involved by psoriasis was 30% for patient A, 40% for B, 35% for C and 60% for D. Total PAH (and pyrene) content of the two coal tar preparations was respectively 28800 (3100) and 470 (104) ppm. The samples were collected at different times after the beginning of therapy (from 12 h after the first application of coal tar to 72 h after the last application; for individual details see Table 3). All patients were under controlled diets, were not exposed to other pharmacological therapies and were instructed on the importance of avoiding passive exposure to cigarette smoke during the time of urinary sample collection.

Individual micturations were collected separately in polyethylene containers previously washed with a few ml of absolute ethanol. Two or more contiguous micturations were mixed in order to obtain samples of at least 500 ml each, and the samples were stored at ~20°C. At the time of use the samples were thawed, filtered (millipore 1.2 μm pore size) and divided into three aliquots to be used for the mutagenesis assay, the determination of total PAH levels and the analysis of 1-OH PYR content. A few ml were set aside for creatinine content determination [15].

Baseline levels of total PAH and mutagenic activity were determined in evening urinary samples of five healthy, non-smoking subjects, not exposed to PAH. The control group for the determination of 1-OH PYR levels consisted of 52 non-smokers.

**Urinary mutagenicity assay**

Samples of 250 ml each were used for the mutagenesis assays after concentration/extraction on XAD-2 resin. From each sample, different amounts of urinary extracts (from 25 to 3.125 and in some cases 1.5625 ml equivalents) were assayed in the Ames plate incorporation assay [25] on S. typhimurium strain TA 98 in the presence of 50 μl/plate of Aroclor 1254 induced liver S9 (protein content 35–38 mg/ml) from Sprague-Dawley male rats and 200 U/plate β-glucuronidase (type VI from Helix pomatia, Sigma). All dose levels were assayed in duplicate, reporting the mean values. An evident reduction in the background lawn of bacteria was evaluated as toxicity of the sample tested, and the corresponding plate was not included in the counts.

The mutagenic activity of different dilutions of each urinary extract was expressed as the number of induced revertants/g of creatinine (spontaneous revertants were subtracted). The highest value obtained was considered as indicative of the mutagenic activity of the corresponding urinary sample. A urinary sample was considered as positive in the assay if the mutagenic activity value of the 95th percentile of the control population was exceeded.

The mean number of spontaneous revertants ± SD per plate throughout all the experiments was 23 (±6) (n = 7). Benzo(a)pyrene (5 μg/plate) was used as a positive control in the presence of 50 μl/plate of S9 (mean number of revertants on strain TA 98 = 504 ± 125, n = 7).

**Determination of total urinary PAH**

After concentration of the urinary samples (100 ml each) on C-18 resin and elution with methanol, the extracted metabolites were reduced and deconjugated in boiling hydrochloric acid as described by Becher and Bjorseth [1]. The resulting compounds were then analyzed by high resolution gas chromatography/mass spectrometry (HRGC/MS) [33].

**Determination of urinary 1-OH PYR**

The method for the determination of urinary levels of 1-OH PYR was developed by Jongeneelen et al. [16]. The determination is based on the enzymatic hydrolysis of the conjugated metabolites, followed by the concentration/extraction of the metabolites. The extracted metabolites are then eluted and the corresponding plate was not included in the counts.

**Results**

The urinary levels of 1-OH PYR, the mutagenic activity and the total PAH content in the eight samples...