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Evaluation of a battery of *Salmonella typhimurium* tester strains for biomonitoring of mutagenic polycyclic aromatic hydrocarbons, nitroarenes and aromatic amines

Anna Nikoyan, Michel De Méo *, Irène Sari-Minodier, Florence Chaspoul, Philippe Gallice, Alain Botta

Laboratoire de Biogénotoxicologie et Mutagenèse Environnementale (EA 1784), Facultés de Médecine et Pharmacie, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France

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Abstract

Various combinations of *Salmonella typhimurium* tester strains and S9 mix for bioactivation (TA98 + S9 mix, TA98S; YG1041 + S9 mix, YG1041S) and strain YG1041 in the absence of S9 mix (YG1041) were used to evaluate the mutagenic activity of eight polycyclic aromatic hydrocarbons (PAHs), seven nitroarenes (NAs) and seven aromatic amines (AAs). Three cigarette smoke extracts and two extracts of smokers' urine (SUE) were also included. Urinary mutagenicity was then determined on 31 individuals, potentially exposed to PAHs, for 0 h, 7 h, 12 h and 24 h. Concentrations of urinary 1-hydroxypyrene (1OHP) and 3-hydroxybenzo[*a*]pyrene (3OHBaP), the levels of atmospheric pyrene (Py) and benzo[a]pyrene (BaP), and particulate concentrations in air (AP) were also measured. PAHs could be detected by TA98S and YG1041S, with TA98S being more sensitive than YG1041S. While NAs could be detected by all combinations, YG1041 and YG1041S were more sensitive than TA98S. Although both YG1041S and TA98S could detect AAs, YG1041S was more sensitive than TA98S. Cigarette smoke extract contained mutagenic AAs and NAs, but AAs were the only mutagenic compounds detected in the extracts of smokers' urine. The concentrations of 1OHP (7 h and 12 h) were significantly higher than those at 0 h, but no difference could be detected with 3OHBaP. Correlations were found between Py and 1OHP (7 h and 24 h) and between BaP and 3OHBaP concentrations (7 h, 12 h and 24 h). A significantly elevated urinary mutagenicity was detected with YG1041S at 7 h in the group of smokers. A good correlation was determined between AP and the test results with TA98S (7 h) and with YG1041 (0 h and 7 h). Urinary 1OHP correlated with the test results with YG1041S (0 h, 7 h and 12 h) while 3OHBaP correlated with those obtained with YG1041S (7 h). Overall, 21/31 individuals were occupationally exposed to AAs, 15/31 individuals were exposed to NAs, and 2/31 were exposed to PAHs as indicated by the *Salmonella* mutagenicity assay. The urine mutagenicity test was not effective at monitoring occupational exposure to PAHs. However, the correlation with AP implied the presence of unknown mutagenic atmospheric substances that could modulate the urinary mutagenicity.

Keywords: Mutagenicity; *Salmonella* assay; Bioactivation; Cigarette smoke extract; Smokers' urine extract; Biomonitoring

1. Introduction

The *Salmonella typhimurium* microsomal assay has long been used to evaluate urinary mutagenicity and to monitor environmental and occupational exposures to

* Corresponding author. Tel.: +33 4 91 83 55 60; fax: +33 4 91 79 86 75.
E-mail address: michel.demeo@pharmacie.univ-mrs.fr (M. De Méo).

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genotoxins [1]. In most studies, this assay has appeared to be useful when the genotoxic agents are components of complex mixtures, when chemical analysis is not available or difficult to perform, and when the suspected genotoxic agent is unknown [2].

In the past, the urine mutagenicity testing has often been used to monitor occupational exposures to polycyclic aromatic hydrocarbons (PAHs). An increase of mutagenicity in urine has been reported in coke-oven workers [3], rubber workers [4], and steel workers [5]. Possible involvement of PAHs has been also reported in subjects exposed to coal-tar pitch volatiles [6], mineral oils [7] and during carbon electrode production [8]. However, the results of PAH monitoring studies where PAHs are the primary toxicants and urinary mutagenicity is used as a biomarker have been inconsistent [9].

The main reasons for discrepancies were the presence of confounding factors such as smoking, mutagenic and/or anti-mutagenic dietary factors and consumption of medical drugs with bactericidal and/or mutagenic effects. Lastly, urinary mutagenicity has been shown to be modulated by polymorphisms of several genes involved in PAH metabolism such as microsomal epoxyde hydrolase [9], glutathione *S*-transferase M1 and *N*-acetyl transferase 2 [10]. Consequently, analytical and molecular-based methods have gradually replaced urinary mutagenicity testing. In addition, the limited specificity for the mutagenic agents due to these confounding factors, the low sensitivity of the technique, the individual variability, the impossibility to evaluate cumulative exposure and the unknown biological significance have significantly hampered the detection of occupational exposure to PAHs [11].

During the last 15 years, the sensitivity of the *Salmonella* microsomal assay has been increased by the use of micro-suspension techniques [12,13]. The sensitivity and the specificity also have been improved by the development of new tester strains such as YG1041 and YG1042 [14]. These strains possess higher levels of nitroreductase and *O*-acetyltransferase and are more sensitive to nitroarenes (NAs) and aromatic amine (AAs) than their corresponding *Salmonella* tester strains (TA98 and TA100). Finally, the evaluation of the mutagenic activity also has been improved by the use of non-linear dose–response models that give more accurate mutagenic potencies than the linear models in the micro-suspension techniques [13,15].

The choice of the different strains is dictated by the compound metabolism. PAHs are converted to arene oxides by cytochrome P450(CYP)-dependent monooxygenases (CYP1A1, CYP1A2, CYP1B1 and CYP3A4). Arene oxides may give phenols by spontaneous isomerisation. However, the microsomal epoxide hydrolase converts arene oxides to *trans*-dihydrodiols. If the latter molecules contain a bay- or fjord-region, they are able to react with DNA to generate bulky adducts [16]. These DNA lesions induce frameshift mutations that are detected by strain TA98 as pro-mutagens in the *Salmonella* microsomal assay.

The metabolism of NAs, especially nitro-PAHs is complex. At least five pathways through which DNA lesions can be induced are possible: (i) nitro-reduction; (ii) nitro-reduction followed by esterification (mainly acetylation); (iii) oxidation; (iv) oxidation and nitro-reduction and (iv) oxidation and nitro-reduction followed by esterification [17]. In bacteria, nitro-reduction is the main metabolic pathway and nitro-reductase over-expressing strains (such as YG1041 for the detection of frameshift mutagens and YG1042 for the detection of base-pair mutagens) are more sensitive than the respective parental strains (TA98 and TA100). For most NAs, no external metabolic activation is required to detect the mutagenic activity of nitroaromatics [14].

AAs and heterocyclic AAs are converted by CYP1A1, CYP1A2, CYP1B1, CYP3A4, CYP3A7, flavine-containing monooxygenases and peroxidases [18]. The resulting *N*-hydroxylamines can be further activated by *N*-acetyltransferase (NAT), sulfotransferases, polyRNA synthetase and kinases to yield *N*-acetoxy, *N*-sulfonyloxy, *N*-prolyloxy and *N*-phosphatyl esters, respectively [19]. These metabolites bind covalently to DNA and can induce frameshift mutations. In the *Salmonella* assay, these molecules have been found to be extremely powerful pro-mutagens that can be preferentially detected by TA98. Strains over-expressing acetyltransferase (YG1041 and YG1042) are more sensitive than the parent strains TA98 and TA100 [14].

The initial approaches to use the urinary mutagenicity for evaluating exposure to PAH led to inconsistent and unsatisfactory results. However, alternative analytical methods were developed to directly measure PAH metabolites in urine [16]. Among the different metabolites of PAHs, 1-hydroxypyrene (1OHP), the main urinary metabolite of pyrene, has been suggested as a biological indicator of PAH exposure [20]. Evaluation of this compound has been shown to reflect the skin absorption of PAHs [9]. However, several disadvantages have been reported. Although 1OHP has appeared to indicate the absorption of pyrene (Py) and other low-molecular weight PAHs, 1OHP is not a direct marker of five-ring PAHs such as benzo[*a*]pyrene (BaP) and dibenz[*a*,*h*]anthracene which are well known carcino-