Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 3 (3): 230-235, 2008
ISSN 1816-496X

Monitoring Ames Assay on Urine of Clinical Pathology Laboratories Technicians

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Abstract: Forty urine samples of clinical pathology laboratory technicians were examined for the presence of mutagenic substance in urine. Mutagenic substance in this study was formaldehyde, formaline, hematoxyline-eosin, Xylole and Xyline which are used for smear fixation and staining in clinical pathology laboratories. We used Ames test to determine the mutagenic potential of above mentioned substances. Mutagenicity was evaluated by TA100 strain of *Salmonella typhimurium*. Urine extracts were prepared using XAD-2 resin in column. The resin was then rinsed with Milli-Q water. This procedure eliminates traces of water soluble growth factors (especially histidine) from the resin. Residual water was removed by vacuum aspiration and adsorbed substance were eluted with a mixture of methanol/acetonitrile v/v. After evaporation to dryness with N_2 gas, the residue was dissolved in DMSO to reach 100 to 250 fold concentration and then urine extraction were kept frozen in liquid nitrogen gas until use. Mutagenicity was evaluated in TA100 *Salmonella thyphimurium* tester stain (overnight cultures) with and without addition of S-9 mix. The results of this study has been shown that 20% of urine samples from technicians of clinical pathology laboratories in Tehran (Iran) were contained mutagen materials. The staff with working history in clinical pathology laboratories may excrete mutagenic compounds in urine.

Key words: Ames assay, TA100 strain, urine samples, clinical pathology laboratories technicians

INTRODUCTION

Considering that cancer is one of the most common and ever increasing diseases in the world and the most of mutagens are also carcinogens (more than 90% of mutagens are carcinogens and vice versa). Several monitoring studies including chlorinated drinking water using vicia faba (Monarca *et al*., 2005), Chlorinated pesticide (Chlorothalonil) using Ames test on urine samples of farmers (André *et al*., 2002), arsenic induced DNA damage using alkaline comet assay (Basu *et al*., 2005), erythrosine and formaldehyde using long-term toxicity/carcinogenicity studies in rats (Borzelleca and Hallagan, 1987; Im *et al*., 2006) have detected the presence of mutagenic activity. The association between the mutagencity of chlorinated drinking water and cancer of the urinary and gastrointestinal tract has been found (Koivusalo *et al*., 1994b, 1995; Schenck *et al*., 1998; Tao *et al*., 1999). Andre *et al*. (2003) have shown that in smoker farmers, chlorothalonil has most frequently mutagenic effect using TA98 with S-9 mix, a strain usually described as able to detect genotoxic metabolites due to tobacco consumption. It has also been demonstrated that chronic exposure to arsenic is associated with increased DNA damage in the exposed subjects (Basu *et al*., 2005). Long-term toxicity/carcinogenicity of erythrosine and formaldehyde have also been demonstrated in rats

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(Borzelleca and Hallagan, 1987; Im *et al*., 2006). In view of the fact that numerous mutagens in different states are present in workplaces such as pathology laboratories, workers in these places not only by skin exposures but also by inhalation can be exposed to these substances. Because of these reasons, identifying and control assays of carcinogenicity in these cases are necessary. With this background we carried out monitoring the Ames test in urine of technicians of clinical pathology laboratories in Tehran/Iran.

MATERIALS AND METHODS

Technicians Characteristics

Urine samples were collected from clinical pathology laboratories technicians of four different clinical pathology laboratories on March 2005 in Tehran/Iran. Technicians use formaldehyde, formaline, hematoxyline-eosin, xylole and xyline for fixing and staining smears in clinical pathology laboratories. Besides providing urine samples, technicians agreed to complete a questionnaire focusing on workplace safety, work history and life conditions characteristics. Data confounding factors such as medication and tobacco consumption and other small interaction factors in formations were also collected in questionnaire. Overall 40 urine samples from 40% male and 60% female partners, aged 25 to 45 (mean 35) year were collected. Some of technicians were smokers. The study was submitted to and approved by the local ethical committee. All technicians gave their informed consent prior to their enrolment in the study.

Collection of Urine Samples

Samples were collected, at evening of the Thursdays for three week and were stored at -20°C until required for extraction.

Preparation of Urine Extraction

Extraction columns were filled 1 g of SAD-2 or Amberlite XAD-2. Resins were conditioned with 5 mL of methanol followed by 50 mL of Milli-Q water for SAD-2, Urine samples were deforested at room temperature and filtrated o Whatman paper No. 1. Solid-liquid extraction was performed on resins by applying the volume of urine (100-250 mL, depending of the total volume available). Resins were then rinsed with Milli-Q water. This procedure eliminated traces of water-soluble growth factors (especially histidine) from the resins. Residual water was removed by vacuum aspiration. Asorbed substances were eluted with a mixture of methanol/acetonitrile (v/v). After evaporation to dryness with speed nitrogen gas, the residue was dissolved in DMSO to reach a 100-250 fold concentration. Urine extracts were kept frozen in liquid nitrogen until use (Andre *et al*., 2002).

S-9 Mix Buffer Preparation

Male wistar rats weighing 200 g were pretreated with phenobarbiturate 50 mg kg^{-1} in drinking water for 5 days. Animal was killed. Liver excised and homogenates prepared in 1.15 mol KCl. The liver microsomal supernatant was obtained by centrifuging the homogenate at 9000 g for 20 min and microsomal suspension was prepared by centrifuging the microsomal supernatant at 105000 g for 60 min and resuspending the pellet KCl. For spectral studies in microsomal pellet was washed once with 1.15 mol KCl. The 105000 g supernatant is refereed to as the soluble fraction. We prepared 5% S-9 mix buffer from liver microsomal supernatant and Co-factors and kept frozen liquid nitrogen until use. Co-factors for S-9 mix are prepared by the methods of Ioannides (1975); Mortelmans and Zeiger (2000).

Culture Medium Preparation

Top agar and Glucose minimum agar and nutrient agar and nutrient broth were prepared and stored at fridge 4°C for future use by according of Mortelmans and Zeiger (2000).

IMViC Test

Some of biochemistry tests evaluated such as Indol, Methyl Red, Voqes-prusquer and citrate on TA100 for enterbacteriacea determination. Some these tests had variations results for TA100 strain. (Edmund, 1977).

Genetic Analysis Tests

It is recommended that the tester strains be analyzed for their genetic integrity and spontaneous mutation rate when frozen cultures are prepared. We used the following tests for a complete TA100 strain check (Biotin and histidine dependence-rf*a* marker-bio-uvrB-plasmid PKM101 for ampicilin resistance) (Mortelmans and Zeiger, 2000).

Ames Assay

Mutagenicity was evaluated by the plates incubated procedure described by Mortelmans and Zeiger (2000) in over night couture of TA100 *Salmonella typhimurium* tester strain with and without S9 mix fraction. For this strain spontaneous background number of revertant and standard deviation are indicated in parentheses, in the absence or presence of S9 mix, respectively: TA100 (160±8, 146±6, 127±3, 150±5). A positive control and two negative controls for TA100 strain in both conditions was systematically used. For each extract, three times 1/100 concentration were tested (brought to constant volume of 0.05 mL): Extracts were diluted with DMSO. In this study amount of sodium azide as positive control without using S-9 mix buffer was 5 micro gram/plate. The amount of 2-amino anthracene as positive control with using S-9 mix buffer was 0.5 micro gram/plate. Average counts of colonies in plates containing DMSO and water distilled considered as negative controls (Mortelmans and Zeiger, 2000).

Aseptic Technique and Safety Procedures

It is important that basic bacteriological laboratory procedures be used to minimize exposure to the Salmonella tester strains. Surface areas must be properly disinfected before and after use. Though wild-type *S. typhimurium* can cause diarrhea and food poisoning. Laboratory safety procedures such as using plugged pipettes and autoclaving all contaminated material (Mortelmans and Zeiger, 2000).

Analyses of Data

All calculations were performed using SPSS. Differences among treatment groups were tested using ANOVA. Differences in which $p<0.05$ were considered statistically significant. In cases where significant differences were detected, specific post hoc comparisons between treatment groups were examined with Student-Newman-Keuls tests.

RESULTS AND DISCUSSION

Urine extracts were tested in the TA100 strain of *Salmonella typhimurium* in the presence and absence of 5% S9 mix. The ratio of sample colony over negative control colony count was 2 or 3 fold in the presence and absence of 5% S9 mix buffer. The results has been shown in Table 1-4. Data are expressed as Mean±SD. The data were analyzed by using of variance (ANOVA-ONE WAY) follow by post hoc comparisons between groups were examined with student Newman-Keuls tests. $p<0.05$ was considered as the significant. On the basis of tables data, ratio values <0.48 are considered as non cigarette smokers and less or no exposed to mutagens.

Significant genotoxic effects have been reported for various molecules with particular strains of *Salmonella typhimurium* or *E. coli* (Hour *et al*., 1998). The study of population under exposure conditions is a useful approach to consider the mutagenicity of variety of substances and is

Table 1: Colony counts results in clinical pathology laboratory A TA100 revertant colony

	With S9 mix Mean±SD		Without S9 mix Mean±SD	
Positive controls: 1-Na-azide	-		385±8	
2-2-Aminoanthracene	408.0±10		-	
Negative controls: 1-DMSO	236.0±6.5		200±10	
2-D.W	184.5±6.0		120±5	
Sample ratio		R_2		R_1
1	190.5±7.5	0.90	40.0±4.0	0.25
2	170.6±8.0	0.80	59.8±3.6	0.37
3	151.0±4.3	0.71	65.9±5.7	0.41
4	122.7±3.4	0.58	57.4±2.0	0.35
5	130.1±9.2	0.61	78.8±8.5	0.49
6	313.7±11.5	1.48	151.9±7.2	0.94
7	352.0±9.2	1.67	251.0±10.7	1.56
8	275.9±8.4	1.30	207.5±7.0	1.29

Table 2: Colony counts results in clinical pathology laboratory B TA100 revertant colony

	With S9 mix Mean±SD		Without S9 mix Mean±SD	
Positive controls: 1-Na-azide	-		0355.4±10.5	
2-2-Aminoanthracene	410.0±9.5		-	
Negative controls: 1-DMSO	218.0±10.0		0168.5±09.5	
2-D.W	158.5±3.4		1124.0±03.0	
Sample ratio		R_2		R_1
1	258.9±7.5	1.37	240.0±4.5	1.64
2	159.4±1.0	0.84	146.6±4.3	0.994
3	97.6±5.2	0.51	50.9±4.0	0.346
4	123.2±7.0	0.65	70.7±3.1	0.488
5	180.0±2.5	0.95	146.5±4.75	0.68
6	67.2±5.1	0.35	50.9±2.0	0.34
7	191.35±3.75	1.01	144.86±4.3	0.99
8	121.6±3.3	0.64	73.0±2.5	0.49
9	237.0±6.5	1.25	110.5±3	0.75
10	159.9±5.0	0.84	89.4±3.25	0.61
11	390.5±11.5*	2.07*	296.0±4.1*	2.02*
12	79.6±4.5	0.42	75.13±2	0.51
13	80.9±3.1	0.42	65.0±2.3	0.44
14	203.0±8.2	1.07	98.7±3.0	0.67

Table 3: Colony counts results in clinical pathology laboratory C TA100 revertant colony

	With S9 mix Mean±SD		Without S9 mix Mean±SD	
Positive controls: 1-Na-azide	-		0360.5±5.0	
2-2-Aminoanthracene	420.0±8.4		-	
Negative controls: 1-DMSO	209.0±7.3		109.5±2.5	
2-D.W	236.0±8.5		134.0±2.9	
Sample ratio		R_2		R_1
1	156.0±5.2	0.70	59.5±1.5	0.49
2	131.0±8.5	0.58	49.9±0.7	0.412
3	180.0±7.0	0.80	126.0±3	1.03
4	112.26±4.0	0.50	108.8±4.75	0.80
5	450.0±5.5*	2.02*	245.6±3.2*	2.02*
6	461.0±10.6*	2.07*	250.0±6.2*	2.05*
7	193.5±4.6	0.86	106.8±7	0.87
8	162.25±7.2	0.72	81.9±4	0.67
9	104.2±3.5	0.46	8 1.4±2.7	0.66
10	192.8±5.3	0.86	131.3±4.6	1.08

successfully used for populations environmentally and occupationally exposed. The relevance of the Ames test in biomonitoring studies is related with type of exposure and the selected salmonella strains (Andre *et al*., 2002). In this study we used Ames test using TA100 with and without S9 mix to

Table 4: Colony counts results in clinical pathology laboratory D TA100 revertant colony

	With S9 mix Mean±SD		Without S9 mix Mean±SD	
Positive controls: 1-Na-azide	-		0360.5±7.0	
2-2-Aminoanthracene	395.0±5.0		-	
Negative controls: 1-DMSO	220.5±7.0		180.7±7.5	
2-D.W	179.5±6.5		125.5±2.75	
Sample ratio		R_2		R_1
1	74.8±3.5	0.37	49.8±1.7	0.32
2	352.5±3.25	1.76	200.0±4.0	1.30
3	233.5±4.5	1.16	102.0±3.7	0.66
4	148.5±3.6	0.74	51.7±2.2	0.33
5	148.32±3.25	0.74	72.8±2.75	0.47
6	216.84±9.8	1.08	105.0±8.5	0.68
7	410.5±8.75*	2.05*	308.67±5.5*	2.01*
8	402.9±6.5*	2.01*	306.25±7*	2.0*
9	184.66±3.4	0.92	110.5±3.0	0.72
10	228.9±6.5	1.14	157.0±4.2	1.02
11	280.9±7.7	1.40	175.5±6.2	1.15
12	345.8±6.9	1.72	266.9±5.5	1.74
13	415.2±11.5*	2.07*	310.9±10*	2.03*
14	157.9±5.7	0.78	99.9±6.25	0.65
15	165.4±5	0.82	103.2±4.4	0.67
16	90.8±3.3	0.45	37.8±1.8	0.24

Persons who excrete mutagenic compounds = *

$$R1, R2 = \text{Ratio}, \ \text{Ratio} = \frac{\text{Sample colony count}}{\text{Negative control colony}}$$

D.W = Sterile distled water , DMSO= Negative control, Organic Solvent

evaluate mutagenicity of substances used in clinical pathology laboratories for smear fixation and staining in urine extracts, To determine the significant variations of the revertant number with the highest sensitivity, we analyzed the results according to two-fold rule and ANOVA one way test. In conclusion, in the present study, we observed mutagenic activity in urine extracts of 20% of technicians of clinical pathology laboratories. The lack of mutagenicity in 80% of technicians my be related to use masks, gloves and avoiding and drinking in laboratory and observing workplace safety. In this study, positive results including results of both tests, with and without S-9 mix and sensitivity of these methods in identification of mutagens considered equal. In other hand, mutagenicity of substances and their metabolites in this experiments are also the same. Considering the contamination of personals with mutagens in this laboratories, observation of health strictly principles in clinical pathology laboratories are recommended.

ACKNOWLEDGMENTS

We are grateful to the technicians for their participation and Department of Pharmacology for technicians support. We acknowledge welfare organization of Tabriz/Iran for financial support of M. Rezai-Basiri.

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