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Mutagenicity in Urine from Nurses Handling Cytostatic Agents

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Abstract—*A cohort study of* 29 *nurses who extensively handle cytostatic drugs,* 29 *controls matched on sex and age, and seven patients under chemotherapy was carried out between* 1983 *and* 1985. *In a first study, urinary mutagenicity assays performed with the Ames test towards* Salmonella typhimurium *TA* 98 *with and without S*9 *mix gave an increased mutagenic activity, although not significant, for nurses as compared to controls, after adjustment on smoking habits. The results of mutagenicity assays for patients were significantly higher* ($P < 0.01$) *than for non-smoker nurses and non-smoker controls with TA* 98 *without S*9 mix. *Of the* 29 *pairs, complementary assays were performed for non-smokers, that is* 11 *nurses and* 11 *matched controls, with TA* 98, *TA* 100 *and TA* 1535 ± *S*9 *mix. A significant increase in mutagenic activity* ($P < 0.05$) *was detected for nurses as compared to matched controls towards TA* 98 *with and without S*9 *mix. Moreover, the mutagenicity was significantly increased* ($P < 0.05$) *in nurses who handled at least one electrophilic agents as compared to nurses who handled non-electrophilic drugs towards TA* 98 *with and without S*9 *mix.*

INTRODUCTION

MANY cytostatic drugs, widely used in cancer chemotherapy, have been shown to be carcinogenic in animals. Some of these drugs, particularly alkylating agents have been associated with second malignant tumors in patients under chemotherapy [1].

Several studies have been performed about possible health hazards in personnel exposed to small amounts of cancer therapeutic drugs, with conflicting results. A significantly increased frequency of sister chromatid exchanges in nurses as compared to controls has been reported [2, 3]; however, this finding has not been confirmed [4, 5]. Concerning chromosomally aberrant lymphocytes, a significant increased frequency of chromosome breaks among nurses has been recently reported [6]. An increase in chromosome gaps frequency, but not in chromosome breaks frequency was also reported [3]. Moreover, both positive and negative results have been reported about urinary mutagenicity and occupational exposure to cytostatic agents [4, 5, 7–12]. The results of these different studies are summarized in a recent paper [13].

These findings prompted us to perform a cohort study comparing urinary mutagenicity in oncology nurses who extensively handle cancer chemotherapeutic agents, patients under chemotherapy and non-exposed controls.

MATERIALS AND METHODS

A cohort study, exposed–non-exposed, was carried out at the Institut Gustave Roussy from 1983 to 1985.

Thirty nurses (28 women and two men, mean age 31.6 yr) working in oncology units agreed to participate in the study. Of them, 10 were smokers. The nurses noted the different drugs they were routinely handling, and for each the number of injectable antineoplastic agents prepared and admixed by week and the duration of exposure. This number of preparations of antitumor drugs prepared and admixed was in average 65 by week, and the mean duration of exposure 4 yr. They wore no gloves or other protective clothing, except

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Abbreviation used: CI = Confidence Interval.

seven nurses who handled methotrexate with gloves. The drugs most frequently handled include 5 fluoro-uracil, cyclophosphamide, vincristine, doxorubicin, vindesine, cisplatin and dacarbazine. A 24-hr urine sample was collected for each nurse after a 3-day work shift, during an off-duty day, from Saturday morning until Sunday morning.

Thirty office clerks of the hospital, matched by sex and age (± 3 yr) were included. Seven were smokers and none had ever had any contact with cytostatic drugs. The 24-hr urine samples were collected after a working week.

Each exposed and non-exposed subject was interviewed on possible exposure to other mutagenic agents. The questionnaire included questions about X-rays, regular medications over the last 5 yr, illnesses over the last 6 months, oral contraceptives, coffee, and hair dycing in addition to smoking. No significant difference of exposure to such agents was found between nurses and controls.

A positive control group consisting of seven non-smoker patients undergoing intensive chemotherapy (principally vincristine, cyclophosphamide, vindesine, bleomycin) at the hospital was included in the study. A 24-hr urine sample was also collected from each of them.

Urine treatment and mutagenicity assays

Each 24-hr urine sample was homogenized. A sample was drawn in order to determine the creatinine level. According to the volume of urine available, for each subject, one to three samples of 200 ml of urine were brought to pH 7 with NaOH 1N, filtered through Whatman No 1 filter paper and treated with the method of Yamasaki and Ames [14]. After passage of each urine sample through XAD-2 resin (flow rate 2–3 ml/min), the resin was washed with 10 ml of distilled water and eluted with 10 ml of acetone. Each eluate was evaporated to dryness at 40° C under nitrogen, and the residue was taken up in 0.8 ml of dimethylsulfoxide and stored at −20° C. Mutagenicity assays were carried out according to Maron and Ames's method [15], using *Salmonella typhimurium* strain TA 98, with and without a metabolic activation system S9 mix (containing per ml cofactors and 50 µl of S9 prepared from livers of adult male Sprague–Dawley rats pretreated with Arocolor 1254). Histidine has not been observed in urine concentrates in high enough amounts to induce bacterial growth in the test system. Moreover, no mutagenic activity was detected with blanks performed with distilled water and treated in the same conditions as urine. For each assay, 50, 100 and 200 µl urine concentrates (corresponding to 12.5, 25.0 and 50.0 ml of urine) were used, and assays were performed twice for each subject. These doses were reduced when cytotoxicity appeared. For patients, lower samples of urine concentrates, as little as 5–50 µl, were used because of the cytotoxicity at 100 and 200 µl. After 2 days in an incubator at 37° C, the revertants were counted without knowledge of cytostatic exposure.

A complementary study was performed only in 11 pairs of non-smokers still working at the Institut Gustave Roussy. Twenty-four-hour urine samples were collected again for nurses after 3 days of exposure to cytostatics, and during a day of work. Each day, the name of cytostatics handled, and for each the number of injectable antineoplastic drugs prepared and administered were collected. The urine treatment was identical to the one of the first study, and mutagenicity assays were performed with *Salmonella typhimurium* TA 98, TA 100 and TA 1535 with and without S9 mix. For each strain, three or more urine concentrates were used, and assays were also performed twice for each subject.

Statistical analysis

The data were analysed by BMDP programs (Biomedical Computer Programs, 1981). The slope of the linear regression was calculated from the two assays for each subject. A positive mutagenic activity was defined when a significant correlation (at 0.05 significance level) between number of His^+ revertants and volume of urine was observed, and otherwise the result was considered negative. The number of induced His^+ revertants/g of creatinine was calculated as:

$$His^+/g\ \text{creatinine} = \frac{His^+/ml\ \text{urine} * \text{Volume urine}/24\ hr}{g\ \text{of creatinine}/24\ hr}.$$

Mutagenicity was expressed as number of His^+/g creatinine for positive urine concentrates, and the value 0 was assigned to negative ones. The urine mutagenicity results were compared using two-way analysis of variance taking smoking status into account (ANOVA program) after a logarithmic transformation of data, or using a permutation test [16].

RESULTS

One nurse was unexposed at the time of urine collection, so the pair (exposed and matched non-exposed) has been excluded. The results of urine mutagenicity are presented in Table 1 for each group of subjects defined. Using a two-way analysis of variance, the geometric mean of His^+ revertants/g creatinine towards TA 98 without S9 mix, was found increased in nurses (2.83, 95% CI : 1.78–4.50) as compared to matched controls

Table 1. Number of His⁺ revertants/g creatinine among nurses, matched controls and patients with TA 98

	− S9 mix		+ S9 mix	
	Nurses	Controls	Nurses	Controls
Nonsmoker nurses and controls	257	215	743	820
	0	0	0	840
	0	180	8899	836
	269	0	0	0
	168	0	716	0
	208	0	612	0
	0	0	3016	0
	0	463	228	863
	0	250	648	290
	170	0	275	414
	522	0	632	341
	297	0	513	867
	0	0	509	0
	0	0	0	576
	0	0	0	1452
	0	0	0	286
	0	198	0	0
Smoker nurses and nonsmoker controls	470	0	10260	0
	395	0	19991	959
	0	0	6671	0
	0	0	3428	0
	0	0	3215	574
Nonsmoker nurses and smoker controls	316	0	0	11570
	0	0	0	2408
Smoker nurses and controls	1427	0	9211	1110
	285	0	2753	490
	0	653	3457	5297
	0	0	2644	14271
	0	0	1554	0
	Patients		Patients	
	13152		28224	
	4092		5551	
	1483		3844	
	0		721	
	0		0	
	0		0	
	0		0	

(1.66, 95% CI : 1.15–2.41). Similarly, with TA 98 with S9 mix, it was increased for nurses (10.62, 95% CI : 6.03–18.73) as compared to controls (7.25, 95% CI : 4.18–12.58). However, the differences were not significant after adjustment on smoking habits. With respect to smoking habits, the geometric mean of His⁺ revertants/g of creatinine with TA 98 + S9 mix was significantly higher ($P < 0.0001$) in urine of smokers (29.90, 95% CI : 22.26–40.34) than in those of non-smokers (5.27, 95% CI : 2.66–10.45) after adjustment on cytostatic exposure. On the contrary, no difference was found with TA 98 without S9 mix between smokers and non-smokers.

Using a permutation test for two independent samples, the mutagenic activity, towards with TA 98 without S9 mix, was significantly higher for patients than for non-smoker nurses ($P < 0.01$), and than non-smoker controls ($P < 0.002$). Similar results were observed towards TA 98 with S9 mix for patients as compared to non-smoker controls ($P < 0.01$).

The results of urine mutagenicity in 11 non-smoker nurses (urine sample collected during a day of work) and 11 matched controls are presented in Table 2. No mutagenic activity was detected with TA 1535 with and without S9 mix and towards TA 100 without S9 mix either in nurses or in controls.

On the contrary, the mutagenic activity was significantly higher ($P < 0.05$) in nurses as compared to matched controls towards TA 98 with and without S9 mix, using a permutation test for matched pairs. With respect to TA 100 plus S9

Table 2. Number of His$^+$ revertants/g creatinine in non-smoker nurses and matched controls with TA 98 and TA 100

	TA 98 − S9 mix		TA 98 + S9 mix		TA 100 + S9 mix	
	Nurses	Controls	Nurses	Controls	Nurses	Controls
	2399	215	3014	820	8754	0
	0	0	0	840	0	0
	0	180	4060	836	1336	784
	0	0	505	0	0	0
	349	0	0	0	0	0
	0	0	0	0	1870	0
	0	0	0	0	0	0
	0	463	0	863	0	2253
	1227	250	1488	290	0	1022
	4827	0	4047	414	0	302
	1510	0	1661	341	0	0
P value	0.05		0.04		NS	

mix, the mutagenic activity was increased, although not significantly, in nurses as compared to controls.

In order to compare the results of urine mutagenicity according to the nature of antineoplastic drugs, the agents handled by nurses the day of urine collection, were classified in two categories: electrophilic (alkylating drugs, nitrosoureas, cisplatin, mitomycin C) or non-electrophilic drugs [17]. Of the 11 nurses, seven have handled at least one electrophilic cytostatic. Table 3 shows a significant increase in number of His$^+$ revertants/g creatinine among nurses handling electrophilic drugs as compared to nurses handling nonelectrophilic drugs towards TA 98 without S9 mix ($P < 0.05$) and TA 98 with S9 mix ($P < 0.01$).

DISCUSSION

It has been suggested that smoking has a potentiating effect on mutagenic activity in urine towards *Salmonella typhimurium* TA 100 in connection with occupational exposure to cytostatic drugs [11]. The author reported a significantly increased mutagenicity in smoking nurses as compared to smoking controls, and no difference between non-smoking nurses and controls. The present study reported different results using mutagenicity assays with strain TA 98 in the presence of S9 mix: that is, both greater mutagenicity in urine of non-smoking nurses than in those of non-smoking controls, and in urine of smoking nurses than in those of smoking controls. So, according to smoking habits, nurses exhibited increased mutagenic activity as compared to controls, although not significantly. The greater mutagenicity among smoking nurses as compared to smoking controls could not be explained by a different smoking behaviour since the mean number of cigarettes smoked per day did not differ significantly in nurses (15.5) and in controls (15.3). Moreover, mutagens in urine as a consequence of smoking could be detected with strain TA 98 in

Table 3. Number of His$^+$ revertants/g creatinine according to cytostatic drugs handled among nurses with TA 98 and TA 100

	TA 98 − S9 mix	TA 98 + S9 mix	TA 100 + S9 mix
Nurses handling electrophilic drugs	2399	3014	8754
	0	4060	1336
	0	0	0
	0	0	0
	1227	1488	0
	4827	4047	0
	1510	1661	0
Nurses handling non electrophilic drugs	0	0	0
	0	505	0
	349	0	0
	0	0	1870
P-value	0.05	0.02	NS

the presence of S9 mix: mutagenic activity in urine from smokers was 6-fold higher as compared to non-smokers. These results are consistent with those previously reported [14]; i.e. the strain TA 98 plus S9 mix is the most sensitive detector of mutagenicity caused by smoking. The presented results in the first study showed a strong effect of smoking which could mask some of the mutagenicity caused by anticancer drugs. In the second study, the smoking factor was eliminated, and the own effect of anticancer drugs could be evaluated.

Optimal timing for urine collection must be considered in the evaluation of urinary mutagenicity in relation to occupational handling of cytostatic drugs. The first findings from a study of Falck revealed a greater mutagenicity in urine collected for nurses shortly following the last exposure after 3 days of work. Several studies were undertaken later on with urine collection taking place in the same way as Falck's or over a period of several days, with sometimes inconsistent results. In our first study, 24-hr urine samples were collected after 3 days of work during an off-duty day, and the lack of a significant increase of the urinary mutagenicity between nurses and controls could have been perhaps explained by a drug elimination taking place essentially during the night before urine collection. Our second study performed in optimal conditions (smoking factor eliminated and urine collection after 3 days of exposure and during a day of work) shows a mutagenic activity significantly increased ($P < 0.05$) for nurses as compared to matched controls towards TA 98 with and without S9 mix. Moreover, the presented results among nurses showed a significant difference in urine mutagenicity according to the nature of cytostatic handled. These results suggest that manipulation of antineoplastic drugs can constitute a health hazard in nurses.

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