Original Article

An Investigation of Oxidative DNA Damage in Pharmacy Technicians Exposed to Antineoplastic Drugs in Two Chinese Hospitals Using The Urinary 8-OHdG Assay*

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Abstract

Objective To investigate oxidative DNA damage in pharmacy technicians preparing antineoplastic drugs at the PIVAS (Pharmacy Intravenous Admixture Service) in two Chinese hospitals.

Methods Urinary 8-OHdG served as a biomarker. 5-Fluorouracil (5-FU) concentrations in air, masks and gloves were determined. The spill exposure of each PIVAS technician to antineoplastic drugs was investigated. Eighty subjects were divided into exposed group I, II, and control group I, II.

Results 5-FU concentration ratios for gloves and masks in exposed group I were significantly higher than those in exposed group II ($P<0.05$ or $P<0.01$). The average urinary 8-OHdG concentrations in exposed group I, control group I, exposed group II, and control group II were 14.69±0.93, 10.68±1.07, 10.57±0.55, and 11.96±0.73 ng/mg Cr, respectively. Urinary 8-OHdG concentration in exposed group I was significantly higher than that in control group I or that in exposed group II ($P<0.01$). There was a significant correlation between urinary 8-OHdG concentrations and spill frequencies per technician ($P<0.01$).

Conclusion There was detectable oxidative DNA damage in PIVAS technicians exposed to antineoplastic drugs. This oxidative DNA damage may be associated with their spill exposure experience and contamination of their personal protective equipment.

Key words: Urinary 8-OHdG; Oxidative DNA damage; Antineoplastic drugs; Occupational exposure; Pharmacy Intravenous Admixture Service

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INTRODUCTION

The widespread use of antineoplastic drugs for cancer treatment has led to concern about possible health risks for healthcare workers involved in the preparation and administration of antineoplastic drugs, which include cytostatic drugs, hormones and antibiotics. Despite their therapeutic effects, many of these drugs are identified by the International Agency for Research

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on Cancer (IARC) as known or suspected human carcinogens[1]. During the past 30 years, numerous guidelines have been issued to protect health care workers in Western countries[2-4]. Although these safety precautions are advanced to reduce worker exposure, recent studies have demonstrated that workplace contamination and occupational exposure continues[5-8].

In addition to workplace contamination with antineoplastic drugs, biomarkers of genotoxic damage have been utilized to monitor occupational exposure to these drugs because many of them are genotoxic by nature[9-10]. Biomarkers endpoints including chromosomal aberrations (CA), micronuclei (MN), sister chromatid exchange (SCE) and DNA damage have been used in many epidemiological studies. A majority of biomarker studies have shown a significant increase in genotoxic effects in healthcare workers who had handled antineoplastic drugs with inadequate or no protection[11-15]. Moreover, many epidemiological studies have evaluated the genotoxic effects of antineoplastic drugs on health care workers who followed recommended guidelines. Most of these studies revealed negative outcomes using MN, SCE and DNA damage biomarkers, although workplace contamination with antineoplastic drugs or uptake of these drugs often occurred[7,16-18]. However, a few studies have found significant genetoxic effects detected using the CA biomarker in pharmacy technicians or nurses who used adequate protective equipment for handling antineoplastic drugs[19].

So far, there are no specific guidelines for the safe handling of antineoplastic drugs in China. However, in recent years the Pharmacy Intravenous Admixture Service (PIVAS) has been developed as a safety precaution by many large-scale hospitals in China. Safety precautions established in the hospitals mainly include isolated preparation rooms, biological safety cabinets (BSC), personal protective equipment (PPE) and handling procedures for antineoplastic drugs. Another function of the PIVAS is to prepare intravenous fluids (e.g. antineoplastic drugs, antibiotics and electrolytes) for all departments in hospitals. However, our earlier study indicated that these protective measures using the PIVAS model, established in the large-scale hospitals, were probably not effective in preventing workplace contamination with antineoplastic drugs[20]. Our earlier study showed that higher 5-fluorouracil (5-FU) concentrations were detected on PPE (gloves and masks) and various surfaces (e.g. BSC, the floor in preparation rooms, office rooms and terraces, the tables in preparation rooms and office rooms) as compared with controls. PIVAS technicians who prepare antineoplastic drugs may be exposed to antineoplastic drugs in two ways: by inhalation of aerosolized drugs or by direct skin contact[21].

There is a lack of knowledge regarding the potential genotoxic effects of antineoplastic drugs in PIVAS technicians, if a risk of workplace contamination with antineoplastic drugs exists. Therefore, it is necessary to investigate these genotoxic effects in PIVAS technicians who prepare these drugs. In the present study, oxidative DNA damage in PIVAS technicians in two Chinese hospitals was investigated using the urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) assay. Urinary 8-OHdG is a biomarker of oxidative DNA damage and the assay uses a noninvasive method[22-25]. Thus, the urinary 8-OHdG assay was used in the present investigation. Because the frequency of use and quantity of 5-FU is highest among the antineoplastic drugs in the two hospitals, the concentrations of 5-FU in workplace air and on PPE (i.e. masks and gloves) were utilized to reflect the external exposure levels to antineoplastic drugs. The spill exposure experience related to antineoplastic drugs for each PIVAS technician was investigated, which indicated the extent of good work practice in the preparation process.

MATERIALS AND METHODS

Subjects

The eighty subjects enrolled in the study were divided into four groups: (1) exposed group I consisting of 20 PIVAS technicians from Hospital I; (2) control group I for exposed group I consisting of 20 controls; (3) exposed group II consisting of 20 PIVAS technicians from Hospital II; and (4) control group II for exposed group II consisting of 20 controls. The two provincial hospitals were located in Zhejiang province, East China. Each PIVAS technician was matched with one control based on their gender, age, and body mass index (BMI). Inclusion criteria required that the exposed technicians had been preparing antineoplastic drugs for at least 6 months. Forty controls were not exposed to antineoplastic drugs or other chemical and physical genotoxic agents. PIVAS technicians who smoked, took vitamins, had received chemotherapy or radiation therapy, or had suffered kidney disease were excluded from the study. General information regarding these subjects is

detailed in Table 1. Differences in gender, age, and BMI between the exposed groups and the corresponding control groups were not significant (P>0.05). Furthermore, differences in gender, age and BMI between two exposed groups were not significant (P>0.05). Exposure time (months) and dosages (admixture/day/person) related to 5-FU in exposed group I were significantly lower than in exposed group II (P<0.05 or P<0.01). All of the participants were informed of the objective of our study and gave their informed consent. The study was approved by the Ethical Committee of the Second Affiliated Hospital at Medicine College, Zhejiang University (No.34, 2010).

Table 1. General Information with Regard To Drug Exposed Groups and Controls (Mean±SE)

Variable	Exposed group I	*t*	Control group I	Exposed group II	Control group II
Male [*n*, (%)]	3 (15)	-	3 (15)	5 (25)	5 (25)
Female [*n*, (%)]	17 (85)	-	17 (85)	15 (75)	15 (75)
Age (years)	25.70±0.44	-	25.55±0.76	24.70±0.31	24.50±0.39
Body mass index	20.05±0.36	-	19.74±0.39	20.13±0.46	20.09±0.32
Exposed months	22.65±2.79[a]	2.62	-	36.00±4.26	-
5-FU admixture/day/person (g)	6.17±0.33[a]	2.71	-	12.47±1.95	-

Notes. [a]P<0.05, as compared with exposed group II.

The outline of the process of drug preparation is as follows: (1) the neck of the ampoule containing the drug for injection (e.g. 5-FU, cisplatin or homoharringtonine) was cut manually with a small grindstone and was broken with a piece of gauze. Then, the drug was moved out from the ampoule bottle and injected into perfusion bags; (2) the drug for injection (e.g. etoposide and taxol) in vials was extracted directly with injectors and injected into perfusion bags. Drug powder (e.g. pemetrexed disodium and oxaliplatin) in vials was dissolved using normal saline and then injected into perfusion bags.

Protective measures involving the PIVAS model taken in the two hospitals included isolated preparation rooms, BSC, PPE, and handling procedures for antineoplastic drugs. All of these drugs were prepared inside the BSC class II with a vertical laminar air flow (BSC-IIA2, Shanghai Shangjing Purification Equipment Co., Ltd., Shanghai, China). The pressure inside the BSC was below atmospheric pressure. The same PPEs were worn by the subjects in the two hospitals, which included double latex gloves (Suzhou Jiale Company, Jiangsu, China), double surgical masks (Yangzhou Huatai Medical Apparatus Company, Jiangsu, China) and C-class protective gowns.

Investigation of Spill Exposure Experience

A questionnaire was designed to investigate the spill exposure experience of each PIVAS technician during a period of 6 months in 2010. The spill events included splashing, breaking and spraying events. A splashing event was defined as drug splashing from an ampoule bottle when the bottle lid was opened or after opening if the bottle was knocked down; a breaking event was defined as complete drug spillage when an ampoule bottle was broken into pieces after being dropped on a surface or was badly cut by a small grindstone; and a spraying event was defined as drug spraying from an injector when the needle was withdrawn from a vial or separated from an injector. The percentage of PIVAS technicians that experienced spill events and the spill frequencies/ person in each exposed group were investigated.

Air Sampling

Short-time sampling was performed according to the occupational health standard (GBZ159-2004) in China. Sampling sites were the inside and outside of the BSC in the PIVAS preparation room. Sampling was conducted before and at the time of drug preparation. Sampling equipment and the absorption liquid were an air sampler (Airchek2000, SKC, Houston, TX, USA) and 10 mL of hydrochloric acid (0.9%), respectively. The flow rate was 1.0 L/min and the sampling time was 15 min. Six air samples were obtained at three different times during a work shift. Air samples were collected continuously over 3 days.

Mask Sampling

The outer masks and inner masks in the two exposed groups were collected immediately after drug preparation so that no contamination with other materials (e.g. the gloves) was possible. The mask samples were packed in clean plastic bags. Unused masks from the same hospitals served as

controls. Mask samples were collected continuously for 3 days.

Glove Sampling

The double latex gloves of the right and left hands in the two exposed groups were collected after drug preparation. The glove samples were packed with clean plastic bags. Unused gloves from the same hospitals were selected as controls. Glove samples were collected continuously for three days.

Urine Sampling

A spot urine sample (100 mL) was obtained from each subject before lunch on the last working day. Urine samples were collected in clean polypropylene containers that had been washed with nitric acid. The samples were frozen at -20 °C until urinary 8-OHdG analysis was performed.

Detection of 5-FU

Mask and glove samples were cut into pieces and infused with 0.9% dilute hydrochloric acid for 2 h prior to analysis. The 5-FU concentrations of air, mask and gloves were detected at a wavelength of 265 nm using an ultraviolet-visible spectrophotometer (UV-2100S, Shimadzu Corporation, Kyoto, Japan) according to the standard of the Chinese Pharmacopoeia. 5-FU concentrations on the mask and gloves were expressed as 5-FU concentration ratios, which were calculated using the following formula: sample 5-FU concentrations/control 5-FU concentrations.

Detection of Urinary 8-OHdG

Urinary 8-OHdG concentrations were determined using a competitive ELISA kit (new 8-OHdG check, Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd., Shizuoka, Japan) according to the manufacturers' instructions[26]. Urinary 8-OHdG concentrations were subsequently adjusted by urinary creatinine concentrations measured with a Picric kinetic kit (Jiancheng Bioengineering Co. Ltd., Nanjing, China).

Statistical Analysis

ANOVA and the chi-square test were used to analyze general information relating to the exposed groups and controls. The Chi-square test was used to analyze each PIVAS technician's spill exposure experience regarding antineoplastic drugs. The 5-FU concentration ratios of PPE and urinary 8-OHdG concentrations of subjects were analyzed using ANOVA, followed by a LSD post hoc test (for equal variances) or Dunnett's T3 post hoc test (for unequal variances). Urinary 8-OHdG concentrations of exposure groups based on leakage events were analyzed using the *t*-test. Pearson correlation was used to analyze the correlation between urinary 8-OHdG concentrations and age, exposure time (months) and spill frequencies per person. When the *P* value was <0.05, the difference was considered to be statistically significant.

RESULTS

Results of Antineoplastic Drug Spill Events

Each PIVAS technician's spill exposure experience with regard to antineoplastic drugs over a 6 month period is shown in Table 2. The percentages of PIVAS technicians involved in splashing, breaking and spraying events in exposed group I were significantly higher than those in exposed group II ($P<0.01$). The percentage (100%) of PIVAS technicians involved in spill events in exposed group I was significantly higher than that (10%) in exposure group II ($P<0.01$). Spill frequencies per technician (2.50±0.30) in exposed group I were significantly higher than those (0.15±0.10) in exposed group II ($P<0.01$).

Table 2. PIVAS Technician Antineoplastic Drug Spill Exposure Experience over a 6 Month Period

Exposed Group	Splashing[a]			Breaking[b]			Spraying[c]			Spill events[d]			Spill Frequencies /Person	
	n	%	χ^2	*n*	%	χ^2	*n*	%	χ^2	*n*	%	χ^2	Mean	*t*
group I	13	65[e]	19.26	11	55[e]	11.91	18	90[e]	28.97	20	100[e]	32.73	2.50±0.30[e]	7.28
group II	0	0	-	1	5	-	1	5	-	2	10	-	0.15±0.10	

Notes. [a]Drug splashing from an ampoule bottle when the bottle lid was opened or the bottle was knocked down. [b]Drug spilling out completely when an ampoule bottle was broken into pieces. [c]Drug spraying from an injector when the needle was withdrawn from a vial or separated from an injector. [d]Including splashing, breaking and spraying events. [e]$P<0.01$, as compared with exposed group II.

Results of 5-FU Concentration Sampling of Air, Masks, and Gloves

The 5-FU concentrations of all air samples were undetectable. Table 3 shows that the 5-FU concentration ratios of the outer and inner gloves (left hand or right hand) in exposed group I were significantly higher than those in exposed group II (P<0.01). The 5-FU concentration ratios for the outer and inner masks in exposed group I were significantly higher than those in exposed group II (P<0.05 or P<0.01). Moreover, the 5-FU concentration ratios of the outer gloves (left or right hand) in exposed group I were significantly higher than those of the inner gloves in exposed group I (P<0.01). The average 5-FU concentrations of mask and glove controls were 0.011±0.0047 mg per mask and 0.023±0.003 mg per glove, respectively.

Table 3. 5-FU Concentration Ratios for The Latex Gloves and Masks of PIVAS Technicians (Mean±SE)

Groups	Samples	*n*	5-Fu concentration ratios	*t*
Exposed group I	Outer gloves of left hand	18	4.91±0.30[bc]	13.08[b], 5.16[c]
	Outer gloves of right hand	18	4.24±0.30[bc]	10.60[b], 6.33[c]
	Inner gloves of left hand	18	2.86±0.14[b]	11.83
	Inner gloves of right hand	18	1.75±0.09[b]	6.50
Exposed group II	Outer gloves of left hand	36	1.29±0.06	-
	Outer gloves of right hand	36	1.29±0.05	-
	Inner gloves of left hand	36	1.21±0.07	-
	Inner gloves of right hand	36	1.21±0.04	-
Exposed group I	Outer masks	14	3.07±0.29[b]	3.54
	Inner masks	14	3.29±0.49[a]	2.59
Exposed group II	Outer masks	36	1.71±0.20	-
	Inner masks	36	1.82±0.31	-

Notes. [a]P<0.05, as compared with exposed group II; [b]P<0.01, as compared with exposed group II; [c]P<0.01, as compared with inner gloves in exposed group I.

Results of Urinary 8-OHdG Concentration Measurement

Table 4 details the average urinary 8-OHdG concentrations in exposed group I, control group I, exposed group II, and control group II. The urinary 8-OHdG concentration in exposed group I was significantly higher than that in control group I and exposed group II (P<0.01). There were no differences in urinary 8-OHdG concentrations among exposed group II, control group II and control group I (P>0.05). Moreover, there was a significant correlation between urinary 8-OHdG concentrations and spill frequencies per person (P<0.01).

DISCUSSION

In the present study, it was found that the antineoplastic drug spill exposure experience of PIVAS technicians could reflect the extent of good work practice that was adhered to during the process of drug preparation. During a 6 month period, 100% of PIVAS technicians in exposed group I experienced spill exposure including splashing, breaking and spraying events, which implied that spill events might often occur in each step of drug preparation. Spill events experienced by PIVAS technicians may be associated with their bad work practice and a likelihood of a lack of proper training. Furthermore, there may be problems in relation to the current PIVAS model with needle technique contributing to drug spills or leakage. Spivey et al.[27] found that each step with the application of a needle technique probably resulted in the leakage of antineoplastic drugs into the environment during the conventional drug preparation system. In addition, spill frequencies per subject in exposed group I were significantly higher than those in exposed group II (P<0.01), suggesting that drug handling by PIVAS technicians in exposed group II was much safer, or more in line with safety standards, than was the case in exposed group I. On the basis of findings from the present investigation, it is clear that PIVAS technician work practice in relation to drug preparation is an important factor in the prevention of exposure to antineoplastic drugs.

Atmospheric concentrations of 5-FU from samples taken in the two hospitals in the present study could not be detected using UV-vis spectrophotometry.

Table 4. Urinary 8-OHdG Concentrations in PIVAS Technicians and Controls (ng/mg Cr)

No	Exposed group I	Control group I	Exposed group II	Control group II
1	22.19	10.02	13.49	8.29
2	16.68	8.29	10.80	24.56
3	12.86	7.20	13.00	17.30
4	12.88	12.17	11.41	12.23
5	19.92	7.34	9.20	7.32
6	18.55	12.98	12.87	11.22
7	10.93	10.18	9.50	8.97
8	13.32	7.02	8.68	17.48
9	18.52	4.03	5.92	9.52
10	10.26	21.76	9.54	9.50
11	17.10	11.16	10.34	17.71
12	9.20	15.52	6.88	7.67
13	11.79	8.14	9.70	12.83
14	11.23	5.62	13.99	13.53
15	9.66	17.60	8.30	14.28
16	14.18	5.49	11.55	12.97
17	19.01	8.20	15.72	9.30
18	14.12	8.02	10.33	12.38
19	10.00	17.81	11.50	14.41
20	21.34	15.11	8.65	13.81
Mean±SE	14.69±0.93[ab]	10.68±1.07	10.57±0.55	11.96±0.73
T	2.84[a], 3.83[b]	-	-	-

Note. [a]*P*<0.01, as compared with control group I; [b]*P*<0.01, as compared with exposed group II.

These results were similar to the results obtained from most previous air-sampling studies, in which significant air concentrations of antineoplastic drugs were not observed[2,7]. In addition, these results suggested that air sampling might not play a significant role in the assessment of exposure to these drugs. However, the significant 5-FU concentrations measured on the outer and inner surgical masks of the two exposed groups were detectable in the present investigation. Sessink et al.[6] also reported that 5-FU (15 μg) could be detected on a mask after a technician had preparation a large quantity of 5-FU (i.e. 21 g per day) even if the new recommended guideline had been adopted. The 5-FU contamination of the inner surgical masks indicated that the surgical masks used in the current PIVAS model were not able to provide PIVAS technicians with adequate protection[2], and probably led to inhalation intake of aerosolized drugs. In the present study, significant 5-FU contamination of outer and inner latex gloves was found in the two exposed groups, which may have led to direct skin contact with antineoplastic drugs. The reason for the contamination of the inner latex gloves was that 5-FU was able to permeate through the surface of the glove[28]. Sessink et al.[29] also reported that 5-FU, cyclophosphamide and methotrexate could permeate through latex gloves and uptake of cyclophosphamide, probably via the dermal route, was observed.

In the present study, higher urinary 8-OHdG concentrations were detected in exposed group I as compared with the controls, indicating that there was detectable oxidative DNA damage in exposed group I, even if the protective measures of the PIVAS model were adhered to. Cavallo et al.[19] reported a significant increase in CA in pharmacy technicians and nurses from an Italian oncology hospital, even though adequate protective measures had been taken. However, in the present study no significant urinary 8-OHdG levels were observed in exposed group II, even if contamination with PPE occurred. It seems that the uptake dose of antineoplastic drugs was not sufficient to induce oxidative DNA damage. This finding was in accord with the results from most of the biomarker studies (e.g. MN, SCE or DNA damage) that revealed the negative outcomes in health care workers who followed the recommended guidelines. For instance, micronuclei (MN) frequencies in 100 German employees from hospitals or pharmacies with central cytostatic drug preparation facilities did not increase significantly as compared with controls[16]. MN and sister chromatid exchange (SCE) frequencies in 13 hospital pharmacists and pharmacy technicians who complied with standard safety precautions did not differ significantly from those of the controls[17]. No DNA damage was detected using the comet assay in 68 health care workers in three US cancer centers after they had followed the recommended safe handling practices, although workplace contamination with antineoplastic drugs occurred in pharmacy and nursing areas[7].

In our study, the urinary 8-OHdG concentrations in exposed group I were significantly higher than those in exposed group II (*P*<0.01). The difference in oxidative DNA damage between the two exposed groups may have been associated with the different spill experience of PIVAS technicians and contamination with PPE. Urinary 8-OHdG levels in PIVAS technicians with more spill exposure experience were much higher than those in PIVAS

technicians with less spill experience. Moreover, urinary 8-OHdG concentrations significantly correlated with the spill frequencies for each technician. Additionally, the results of our study showed that urinary 8-OHdG levels in exposed group I with higher contamination of PPE were much higher than those in exposed group II with lower contamination of PPE.

Urinary 8-OHdG levels may be influenced by many factors, such as renal impairments[30], BMI[31], gender, age, smoking, and taking vitamins[32]. In the present study, these confounding factors were well controlled. All participants had no history of kidney diseases and did not smoke or take vitamins. There were no significant differences in BMI, gender and age between exposed groups and controls. A limitation of the present study was that antineoplastic drug levels in blood or urine were not measured. Thus, a direct correlation between the observed oxidative DNA damage and the internal exposure dose of antineoplastic drugs could not be analyzed. Many studies have shown that significant increases in antineoplastic drug levels in urine or blood were still observed in pharmacy technicians or nurses although they used adequate protective measures for handling antineoplastic drugs[7,16-19]. In our study, 40 PIVAS technicians were selected for inclusion in the exposed groups. The number of PIVAS technicians was 90% of the total PIVAS technicians in the two hospitals, which was greater than the number of pharmacy staff in other similar investigations[7,16-19]. The present study only focused on the genetic damage of PIVAS technicians in the two hospitals, hence, the results of the study cannot be directly extrapolated to all similar hospitals.

In conclusion, our finding indicated that there was detectable oxidative DNA damage in PIVAS technicians exposed to antineoplastic drugs who had adopted poor working practices. Oxidative DNA damage in PIVAS technicians may be associated with the spill exposure experience of PIVAS technicians and contamination of personal protective equipment. The protective measures in the current PIVAS model should be improved as follows: (1) by the development of specific guidelines for the safe handing of antineoplastic drugs; (2) by the use of effective PPE, e.g. wearing respirators instead of surgical masks; (3) by improvement of the PIVAS technician's good working practice by means of training and education programs; and (4) by the establishment of medical surveillance programs for genetic damage in PIVAS technicians.

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