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Determination of the vaporization of solutions of mutagenic antineoplastic agents at 23 and 37°C using a desiccator technique

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Abstract

This study evaluated the ability of mutagenic antineoplastic agents to vaporize at room temperature (23°C) and 37°C. A bacterial mutagenicity assay was used to determine the mutagenicity of these agents in the vapor phase. Open plates of bacteria were exposed to varying amounts of drug solutions in sealed glass containers for 24 h. The drug solutions were prepared as they would be for patient treatment and were tested at 0.25, 0.5 and 1.0 ml of each drug solution per 10 l of air. Following exposure, the plates exposed at 23°C were incubated an additional 48 h at 37°C to allow for expression of mutations. Those exposed at 37°C were incubated for an additional 24 h at 37°C. Carmustine, cyclophosphamide, ifosfamide, thiotepa, and mustargen demonstrated vaporization at 37°C. Carmustine and mustargen also demonstrated significant vaporization at 23°C, while cyclophosphamide demonstrated a 50% increase in revertants at this temperature. In addition, sodium azide, a known mutagen used as a control was also mutagenic as a vapor at both temperatures. Doxorubicin, cisplatin, etoposide, 5-fluorouracil and mitomycin were not detected as vaporizing in this assay. The study found that vaporization of standard solutions of some antineoplastic agents is possible at room temperature and increases as the temperature increases. Therefore, vaporization of spilled antineoplastic agents may present an additional route of exposure to healthcare workers through inhalation. © 2000 Published by Elsevier Science B.V.

Keywords: Antineoplastic agents; Vaporization; *Salmonella*; Occupational exposure

1. Introduction

The toxic side effects of antineoplastic agents are well documented in patients and a number of these effects have been identified in healthcare workers who handle these agents [1–4]. In patients, side effects include toxicity to most organs and tissues, especially those with high turnover rates. Additionally, a growing number of the antineoplastic agents are listed as known human carcinogens, while others are considered probable or possible human carcinogens [2–6]. Genotoxic effects and adverse reproductive outcomes have also been documented in healthcare workers exposed to these agents [1–4,7–9]. Although studies in Europe have shown that contamination and exposure exists in other countries [10–17], we have recently reported that pharmacies and treatment areas in cancer centers in North America are also contam-

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inated with antineoplastic agents despite the use of biological safety cabinets and other protective equipment [18]. Our study documented a higher percentage of samples demonstrating contamination in both the pharmacy and ambulatory areas than a previous study conducted in a single US pharmacy [19]. It has been assumed that exposure of personnel has been through oral and dermal contact and the inhalation of particulates. However, based on reports of the measurement of the vapor pressure of several antineoplastic agents [20,21], the present study was undertaken to determine if inhalation of vapors of spilled agents could be a potential route of exposure for healthcare workers.

Studies have assayed the mutagenicity of vapors of a variety of chemical compounds [22–28], complex mixtures [29,30], and solid agents [31,32]. Typically, these assays have incorporated a sealed glass container with open plates of bacteria for varying exposure times or a procedure where the test agent is placed on the cover of the inverted petri dish and the tops and bottoms of the dishes are sealed together. At a specified time, the exposure to the agent is terminated and the plates are incubated for the required amount of time.

In the present study, a sample of antineoplastic agents in solution was assayed for their ability to vaporize at room temperature and 37°C using a desiccator technique. Two agents, carmustine and cyclophosphamide, were assayed at additional temperatures. A *Salmonella* mutagenicity assay was used to estimate the vaporization of these drugs. The results of the study indicate that 5 of the 10 antineoplastic agents tested vaporized at 37°C and two and possibly a third vaporized at 23°C. These finding may have implications concerning the techniques currently employed in their handling in the healthcare setting and possibly in research laboratories where these agents are used.

2. Materials and methods

Ten antineoplastic agents, carmustine, cyclophosphamide, ifosfamide, doxorubicin, thiotepa, mustargen, mitomycin, cisplatin, etoposide, and 5-fluorouracil were prepared according to the manufacturers' recommendations (Table 1). One reference mutagen, sodium azide, was employed as a control for the testing procedure. *Salmonella typhimurium* strains TA98 and TA100 were supplied by B.N. Ames, University of California at Berkeley. Strains UTH8413 and UTH8414, repair proficient complements of TA98 and TA100, respectively [33], were supplied by T.S. Matney, University of Texas-Houston. Plastic petri dishes containing 20 ml Vogel–Bonner minimal media were plated with a sensitive strain of *S. typhimurium* [34,35] and placed uncovered and right-side-up in 101 glass desiccators that had been equilibrated to the test temperature for several hours before the start of the exposure. The strains were selected based on previous studies in this laboratory with these antineoplastic

Table 1
Antineoplastic agents evaluated in the vapor study

Drug	CASRN	Supplier	Lot number	Amount of drug (mg/ml)	Vapor pressure[a] (mPa)	
					25°C	40°C
Carmustine	154-93-8	Bristol Laboratories, Princeton, NJ	LDS97	3.33	46	530
Cyclophosphamide	6055-19-2	Mead Johnson, Princeton, NJ	G8000A	20	4.4	9.0
Ifosfamide	3778-73-2	Mead Johnson, Princeton, NJ	KGN83	50	1.05	1.2
Doxorubicin	25316-40-9	Fujisawa, Healthcare Inc., Deerfield, IL	180210	2		
Etoposide	33419-42-0	Bristol Laboratories, Princeton, NJ	9E11195	20	2.65	3.8
Thiotepa	52-24-4	Immunex Corp., Seattle, WA	425-205	10		
Mustargen	55-86-7	Merck & Co. Inc., West point, PA	0369J	1.0		
Mitomycin	50-07-7	Bristol Laboratories, Princeton, NJ	9B15275	0.5		
Cisplatin	15663-27-1	Bristol Laboratories, Princeton, NJ	M8F22A	1.0	1.9	3.1
5-Fluorouracil	51-21-8	Pharmacia Inc., Kalamazoo, MI	FFA149	50	2.0	3.9
Sodium azide[b]	26628-22-8	Aldrich Chemical Co., Milwaukee, WI	N/A	N/A		

[a] References [20] and [21].
[b] A mutagenic compound used as a standard control for the test system.

agents [36]. Three plates were placed on a perforated shelf approximately 6.5 cm above the bottom of the desiccators. A volume of 1, 0.5 or 0.25 ml of each drug solution was placed in glass dishes in the bottom of the desiccators and the desiccators were immediately sealed. One desiccator was prepared with no drug to serve as a control at each temperature. The desiccators at room temperature (23 ± 1°C) were maintained in incubators with the power turned off to reduce fluctuations in temperature.

The plates were removed from the desiccators after 24 h and incubated for an additional 48 and 24 h for the 23 and 37°C exposures, respectively, to allow for growth of the bacterial colonies. Because cyclophosphamide and ifosfamide require metabolism to be biologically active [34,37–39], a rat liver homogenate prepared from Aroclor-induced male Sprague Dawley rats (Harlan, Houston, TX) or purchased (Molecular Toxicology Inc., Boone, NC) was added to the plates for these agents. Following the exposure and incubation periods, the plates were scored for the number of revertant colonies. Although only mutagenic agents will produce an effect in the *Salmonella* assay, 5-fluorouracil is toxic to the bacteria at low concentrations [36] and inhibition of bacterial growth was used as the endpoint for this agent. All data points were performed in triplicate and both drugs and bacteria were protected from exposure to UV light.

Additional studies were conducted with carmustine and cyclophosphamide at temperatures between 23 and 37°C due to concerns about possible degradation of these drugs at the higher temperature. Carmustine was assayed at 26, 30, and 34°C, while cyclophosphamide was assayed at 30°C.

Dose–response curves were run concurrently for each drug by adding the drug directly to the plates along with the same strain of bacteria (and S-9 as required) employed in the vaporization study.

3. Results

Of the 10 antineoplastic agents, five — carmustine, cyclophosphamide, ifosfamide, thiotepa and mustargen — demonstrated considerable vaporization at 37°C as evidenced by the increased number of revertant colonies on the plates (Table 2). Additionally, carmustine and mustargen demonstrated somewhat lower levels of vaporization at 23°C. Carmustine, when tested at temperatures between 23 and 37°C, demonstrated the highest level of vaporization at 30°C (Fig. 1). The number of revertants per plate was in the range of 700–1000 for two independent assays. Cyclophosphamide produced a minimal response (approximately 50% above background) at 23°C using two sources of S-9. At 30°C, cyclophosphamide produced a twofold increase in revertant in two separate assays (Fig. 2). None of the other four drugs, doxorubicin, cisplatin, etoposide, and mitomycin, demonstrated any detectable vaporization at either temperature. However, the dose–response data indicated that all agents produced significant mutagenicity (greater than twofold) at both 23 and 37°C (Table 3). With the plate incorporation assay, 5-fluorouracil inhibited growth at all concentrations at both temperatures, but no effects were seen at either temperature in the vaporization study. Sodium azide was equally mutagenic at both 23 and 37°C and demonstrated mutagenicity at concentrations approximately 1000-fold lower than the antineoplastic agents.

4. Discussion

A desiccator or similar exposure techniques have been used to evaluate the mutagenicity of various volatile compounds, mixtures containing volatile compounds, and some solid compounds [22–32]. Vaporization of standard solutions of some antineoplastic agents is evident at ambient temperatures and increases as the temperature increases. The measurement of vaporization at 23°C may be an underestimation of the actual vaporization due to the fact that bacteria, and especially liver enzymes, function more efficiently at 37°C. However, plate incorporation assays were run at both temperatures to control for any effects of temperature. Also, the ability of an agent to diffuse into the agar may affect the mutagenicity of the agent. Studies in this laboratory have shown that etoposide and doxorubicin do not diffuse well in agar, but are detected in the plate incorporation assay (data not shown). The high control values for mustargen may have been due to vapors escaping into the atmosphere of the incubator and affecting the bacteria in the control plates after they were removed from the desiccators for the second 24 h of incubation. Such

Table 2
Mutagenicity of antineoplastic agents tested as vapors at 23 and 37°C

Drug	Strain	Amount of drug (mg/101)	Revertants per plate (S.D.)[a]	
			23°C	37°C
Carmustine	TA100	0	92 ± 6	101 ± 9
		0.84	118 ± 6	379 ± 55
		1.67	224 ± 29	442 ± 106
		3.33	223 ± 36	510 ± 14
Cyclophosphamide	TA100[b]	0	99 ± 24	112 ± 14
		5	118 ± 10	229 ± 16
		10	155 ± 28	242 ± 8
		20	104 ± 14	346 ± 17
Ifosfamide	TA100[b]	0	97 ± 2	85 ± 10
		12.5	97 ± 9	182 ± 8
		25	116 ± 7	274 ± 26
		50	88 ± 5	442 ± 15
Thiotepa	TA100	0	86 ± 22	93 ± 6
		0.25	122 ± 8	362 ± 44
		0.5	108 ± 2	530 ± 37
		1.0	119 ± 29	753 ± 65
Mustargen	TA100	0	128 ± 21	170 ± 113
		0.25	591 ± 73	430 ± 31
		0.5	381 ± 55	532 ± 11
		1.0	638 ± 38	642 ± 99
Doxorubicin	TA98	0	14 ± 4	14 ± 4
		0.5	15 ± 2	10 ± 3
		1.0	12 ± 3	10 ± 2
		2.0	16 ± 3	13 ± 4
Cisplatin	UTH8414	0	19 ± 2	13 ± 1
		0.25	25 ± 2	21 ± 4
		0.5	17 ± 5	12 ± 3
		1.0	20 ± 6	13 ± 4
Etoposide	UTH8413	0	28 ± 4	13 ± 2
		5	26 ± 4	16 ± 3
		10	28 ± 5	19 ± 3
		20	35 ± 3	12 ± 4
Mitomycin	UTH8414	0	18 ± 3	11 ± 3
		0.25	18 ± 7	15 ± 2
		0.5	18 ± 4	13 ± 7
		1.0	19 ± 3	12 ± 1
5-Fluorounacil	TA100	0	84 ± 10	86 ± 4
		12.5	76 ± 12	70 ± 4
		25	85 ± 9	80 ± 1
		50	80 ± 14	97 ± 0
Sodium azide	TA100	0	86 ± 6	75 ± 11
		0.0125	541 ± 53	491 ± 71
		0.025	859 ± 55	844 ± 117
		0.05	944 ± 97	1000+

[a] Mean ± standard deviation of three replicate plates.
[b] Aroclor-induced rat liver S-9 added for metabolic activation.

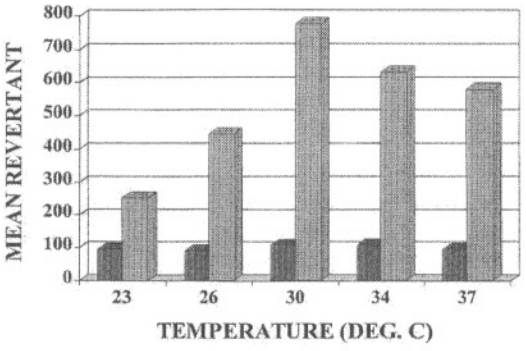

Fig. 1. Vaporization of carmustine at various temperatures. Each assay was conducted in triplicate using 1.0 ml (3.33 mg) of carmustine per 10 l. The values are the means for two separate assays using strain TA100. Plates were exposed for 24 h at the temperatures indicated followed by a 48 h incubation at 37°C, except for those exposed at 37°C which were incubated for an additional 24 h.

a phenomenon with cross contamination by vapors has been seen with sodium azide (E. Zeiger, pers. commun.).

Studies with carmustine at several temperatures indicated the highest response was at 30°C. Carmustine decomposes near its melting point (30.5–32°C) [40] and the decrease in the effect at higher temperatures may be a function of its decomposition. Cyclophosphamide also produced the highest number of revertants at 30°C and it has been reported that hydrolysis of cyclophosphamide takes place at temperatures above 30°C [38]. Although lower concentrations of cyclophosphamide require metabolism to be mutagenic [34,37,38], it appears that, at very high doses, metabolism may not be necessary [39]. It is not known if this effect is due to traces of a decomposition product.

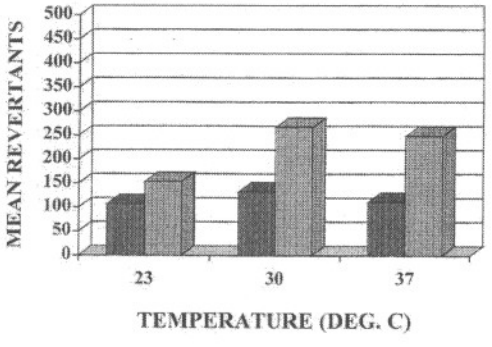

Fig. 2. Vaporization of cyclophosphamide at various temperatures. Each assay was conducted in triplicate using 0.5 ml (10 mg) of cyclophosphamide per 10 l. The values are the means for two separate assays using strain TA100 with S-9 from Aroclor-induced Sprague Dawley rats. Plates were exposed for 24 h at the temperatures indicated followed by a 48 h incubation at 37°C, except for those exposed at 37°C which were incubated for an additional 24 h.

Vapor pressure defines the equilibrium partial pressure between a pure compound and its vapor, and is a function of the substance and of its surrounding temperature. When comparing two compounds at a given temperature, the substance with the larger vapor pressure will have a greater gaseous partial pressure and will partition into the vapor phase to a greater extent.

Limited vapor pressure data are available for the antineoplastic agents studied here (Table 1) [20,21]. However, for three of the five compounds with measured vapor pressures, carmustine, cyclophosphamide, and 5-fluorouracil, the vapor pressure data corroborate their ability to enter the vapor phase as seen in the present study. At 40°C, the vapor pressure of carmustine exceeds that of cyclophosphamide, which exceeds that of 5-fluorouracil following the data that show vaporization of carmustine and cyclophosphamide but not 5-fluorouracil. Further, the vapor pressure of carmustine at 20°C exceeds that of cyclophosphamide at 40°C (which naturally is greater than the vapor pressure of cyclophosphamide at 20°C) explaining why carmustine is seen to vaporize at room temperature, whereas cyclophosphamide vaporization at this temperature may be minimal. However, two agents, cisplatin and 5-fluorouracil, have higher vapor pressures at 40°C (3.1 and 3.9 mPa, respectively), but were not detected in this assay.

Most pharmacies contain a number of heat sources such as compressor motors, lights, pump motors, incubators and others, which could raise the temperature of spills and/or particulates sufficiently so that they could enter the vapor phase. If vaporization of solutions of antineoplastic agents is a significant source of exposure for healthcare workers who handle these agents, precautions should be taken to reduce spills and aerosols to the greatest extent possible. Because the molecule size of vapors is considerably smaller than particulates, biological safety cabinets would not be able to remove them from the atmosphere [41]. This is a serious concern for biological safety cabinets that

Table 3
Mutagenicity of antineoplastic agents tested in a plate incorporation assay at 23 and 37°C

Drug	Strain	Amount of drug (mg/10 l)	Revertants per plate (S.D.)[a]	
			23°C	37°C
Carmustine	TA100	0	82 ± 14	90 ± 9
		10.5	192 ± 22	314 ± 15
		21	319 ± 29	514 ± 60
		42	504 ± 29	703 ± 44
Cyclophosphamide	TA100[b]	0	115 ± 5	116 ± 14
		125	286 ± 20	450 ± 19
		250	512 ± 54	603 ± 60
		500	718 ± 27	855 ± 45
Ifosfamide	TA100[b]	0	94 ± 9	106 ± 13
		125	156 ± 7	186 ± 24
		250	209 ± 18	250 ± 17
		500	255 ± 11	374 ± 34
Thiotepa	TA100	0	101 ± 5	93 ± 7
		125	252 ± 20	477 ± 16
		250	400 ± 35	690 ± 36
		500	626 ± 10	944 ± 50
Mustargen	TA100	0	126 ± 10	155 ± 43
		125	273 ± 42	182 ± 32
		250	337 ± 35	210 ± 43
		500	558 ± 100	242 ± 28
Doxorubicin	TA98	0	18 ± 4	15 ± 6
		3.125	347 ± 28	547 ± 115
		6.25	656 ± 28	1000+
		12.5	645 ± 155	1000+
Cisplatin	UTH8414	0	15 ± 2	14 ± 5
		1.25	NT[c]	180 ± 8
		2.5	381 ± 46	375 ± 38
		5	694 ± 50	667 ± 80
Etoposide	UTH8413	0	17 ± 2	13 ± 2
		250	49 ± 7	46 ± 5
		500	71 ± 12	NT
		1000	96 ± 14	101 ± 6
Mitomycin	UTH8414	0	17 ± 5	14 ± 4
		0.25	36 ± 3	35 ± 9
		0.5	39 ± 6	53 ± 2
		1.0	63 ± 3	86 ± 11
5-Fluorounacil	TA100	0	87 ± 3	86 ± 7
		1.5	T[d]	T
		39	T	T
		78	T	T
Sodium azide	TA100	0	86 ± 2 6	73 ± 15
		0.625	391 ± 37	673 ± 33
		1.25	704 ± 62	1000+
		2.5	1000+	1000+

[a] Mean ± standard deviation of three replicate plates.
[b] Aroclor-induced rat liver S-9 added for metabolic activation.
[c] Not tested.
[d] Reduced background lawn or complete toxicity.

exhaust back into the pharmacy or laboratory through a second HEPA filter. Thus, their potential to be inhaled and absorbed would be considerably greater than that of particulates.

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