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Mutagenicity of Antineoplastic Drug Residues Treated in Health Care Waste Autoclave

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Antineoplastic drugs are common agents in the therapy against many forms of human cancer and some non-neoplastic diseases. In hospitals these drugs are most often used in oncology or radiotherapy units. Human exposure to these agents has been reported in pharmacies and cancer centers treatment areas in United States and Europe (Sorsa et al.1985; Sessink et al. 1992; Hessel et al. 2001), despite the use of biological cabinets and other protective equipment (Connor et al. 1999).

Antineoplastic drugs are recognized as an insidious source of environmental mutagens (Benedict et al. 1977; Kummerer and Helmers 1997). A growing number of antineoplastic agents are listed as known human carcinogens (Ferguson and Pearson 1996). Significant quantities of waste materials containing these mutagenic agents are generated through manufacturing, research, therapy and patient excretion.

The development of new or modified medical waste treatment methods utilizing heat, chemicals or irradiation has provided potential alternative solutions to the health care waste treatment methods. The concern has arisen that these new technologies may also lead to potential environmental or occupational health and safety exposures (EPA, 1994).

The safe disposal of health care wastes has become an ever-increasing concern of the government in Argentina during the last years. In Buenos Aires city area, hospital waste are usually treated in large-scale autoclave (i.e., saturated steam under pressure) to minimize the health risk associated with their handling and final disposal. Autoclaved hospital wastes have low environmental impact, however this methodology is not always suitable for the treatment of chemical or pharmaceutical wastes. After treatment hospital wastes are buried in municipal solid waste landfills and, in some cases, disposed in dumps. In Argentina contaminated material from cytotoxic drug preparation and administration such as contaminated syringes, needles, gauges, vials, packages, etc., are frequently discarded together with the rest of health care wastes. Therefore, the assess of mutagenicity after autoclaving treatment is indispensable because the effect of this process on antineoplastic drugs is unknown and it could be a source of mutagens in the environment. In the present study, the mutagenicity of samples of antineoplastic agents before and after autoclaving treatment were tested with the

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Salmonella mutagenicity assay. The antineoplastic drugs assayed were selected between the most widely used in cancer therapy. The lack of in-depth information on the effects of different hospital waste treatments on antitumor agents points to an obvious need for research to develop this important baseline set of data.

MATERIALS AND METHODS

The antineoplastic drugs cisplatin, carboplatin, doxorubicin, 5-fluorouracil, methotrexate and cyclophosphamide were obtained from Filaxis S.A., Argentina. Solutions were prepared with distilled water. The doses of antineoplastic drugs were adjusted to a range that produced a linear mutagenic response below the toxic range. Two reference mutagens, sodium azide and 2 aminofluorene were employed as positive mutagenesis controls.

Dr. Bruce N. Ames (University of California, Berkeley, CA USA) kindly provided *Salmonella typhimurium* strains TA98 and TA100. All strains were maintained as a frozen stock at –80°C. Confirmation of the genetic integrity of the strains was made according to the methods of Mortelmans and Zeiger (2000). The standard plate incorporation method described by Maron and Ames (1983) was used in all experiments. Negative controls containing the bacteria and solvent ,but not the test chemical, were included in each assay. Revertant colonies were scored following a 72 h incubation at 37°C. The number of colonies scored in the control plates was not deducted from the colonies scored in the test plates. Phenobarbital and β- naphthoflavone-induced rat liver S9 fractions for microsomal activation of compounds were used according to Maron and Ames (1983). All experiments were performed with three plates per concentration.

A large-scale autoclave for hospital waste (Eccotech S.A., model VAP 501) was used during this evaluation. The time-temperature and presion profile of the autoclave cycles were determined with two calibrated temperature probes (model BM 210 type J with digital display) and a pressure indicator (Cimpa Company). For biological control of efficacy of the process, two vials with spores of *Bacillus stearothermophilus* (ATCC 7953) were included. A chemical control was also performed (VaporLine Extended, by Propper Manufacturing Co., Inc.).

Samples of antineoplastic drugs were included in an open glass tube. Autoclave processing time was preset, as a common cycle of treatment, for 35 min with 3 kg/cm^2 of pressure after initial vacuum and injection of steam. Temperature during processing was 141-143°C in all cycles.

After final vacuum and equalization with the atmospheric pressure, the autoclave was opened. Samples and biological controls were removed immediately and submitted to the laboratory for analysis.

The sample with cyclophosphamide was conserved six months after autoclaving, at daylight and room temperature. Mutagenic activity of this sample was tested monthly to detect potential persistence of mutageniciy.

RESULTS AND DISCUSION

Health care waste can be adequately disinfected by autoclaving at 141-143°C (3 kg/cm^2 of pressure) for 35 minutes with a good efficiency on the reduction of spores of *B.stearothermophilus*. During autoclaving there exist a possibility that decomposition or hydrolysis of antineoplastic compounds could generate mutagenic derivatives.

The results obtained when 5-fluorouracil, doxorubicin, cisplatin, carboplatin, methotrexate and cyclophosphamide were tested with the *Salmonella typhimurium* assay are shown in Table 1. Doxorubicin, 5-fluorouracil, cisplatin, carboplatin, did not show any variation in mutagenic activity after autoclaving.The same concentration-response curves were found before and after treatment.

Methotrexate was shown to be nonmutagenic in the Ames test and autoclaving did not generate mutagenic products. Similar results were obtained when the methotrexate was tested with *Saccharomyces cerevisiae* D7 conversion and reversion assay and *Bacillus subtillis* Rec assay (data not shown)

Surprisingly cyclophosphamide showed a variation in its mutagenic effect after autoclaving. Before treatment, its mutagenicity was only detected with TA 100 strain in the presence of the S9 fraction. After treatment the drug was still mutagenic on TA 100 strain with or without S9 activation. Its mutagenic potency was increased by a factor of 5. Unlike the other drugs, cyclophosphamide presents a low melting point 49.5-53.0°C (Castegnaro et al. 1985) and it is sensible to moisture. Hydrolysis and decomposition occur when heated between 60 and 90°C, following a first-order rate of reaction. An initial intramolecular alkylation, followed by subsequent hydrolysis produces several products developing a complex mixture (Masaharu et al 1967; Friedman et al. 1965; Brooke et al. 1975). The mixture formed after autoclaving treatment proved to be more mutagenic than the original compound. The metabolic activation with S9 mix did not show further increases in mutagenicity after treatment.

Since health care waste treated by autoclaving and trituration is finally disposed along with municipal waste in a landfill, the persistence of mutagenic contaminants must be kept in mind. When the treated sample of solution of cyclophosphamide were assayed during six month, the mutagenicity did not demonstrate variation (Figure 1). The rest of antineoplastics assayed conserve their mutagenic potency after treatment.

In municipal landfills this mutagens are mixed with many other biological and nonbiological components with unknown results. Mutagens in landfills can leach through the soil contaminating groundwater, spreading the deleterious effects over vast regions. Antineoplastic drugs must be incinerated and considered as special waste within hospital, and should never be disposed with the rest of health care waste (Pruess et al. 1998).

Table 1. Mutagenicity test of antineoplastic drugs before and after treatment.

	mM	Number of revertants							
		Before treatment				After treatment			
		TA98		TA 100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
FU	0.384	216±12	205±4	t	t	210±14	219±8	t	t
	0.192	190±17	185±14	t	t	186±17	186±17	t	t
	0.038	37±6	40±6	136±8	144±10	35±9	38±5	153±26	141±6
	0.000	37±8	40±4	172±33	161±10	37±8	40±4	172±33	161±10
Do	0.018	280±9	274±8	150±8	171±6	276±4	266±6	153±5	162±2
	0.009	145±6	153±8	150±8	136±2	134±5	141±11	146±6	163±5
	0.002	98±11	103±12	143±11	148±5	104±6	97±3	141±6	148±8
	0.000	22±3	24±1	149±4	141±6	22±3	24±1	149±4	141±6
Cis	0.167	80±1	84±4	720±20	717±13	88±4	87±4	747±18	728±12
	0.083	58±5	60±5	355±4	366±5	62±8	65±6	361±7	390±12
	0.017	44±2	42±3	169±11	170±7	44±2	45±2	178±13	165±17
	0.000	37±7	40±3	162±3	170±12	37±7	40±3	162±3	170±12
Car	1.347	59±5	64±2	270±18	282±10	61±1	61±2	288±15	299±8
	0.673	52±6	51±2	128±8	138±5	53±8	47±2	138±9	148±15
	0.135	37±9	31±8	121±3	125±4	36±6	32±9	118±5	125±4
	0.000	35±3	32±2	125±8	124±4	35±3	32±2	125±8	124±4
Mt	110.0	34±3	36±2	141±3	171±1	40±2	38±3	142±2	156±7
	55.0	31±1	32±0	149±7	176±4	28±2	39±6	142±7	147±15
	11.0	35±3	30±8	137±7	145±6	30±7	30±6	141±3	137±6
	0.0	34±1	35±2	133±7	144±6	34±1	35±2	133±7	144±6
Cp	358.2	36±1	38±5	126±2	609±26	32±2	43±1	3293±78	3217±64
	179.1	23±2	33±3	163±3	435±36	32±2	35±4	1957±31	1923±24
	35.8	24±1	31±1	145±1	257±43	27±3	30±2	981±9	966±44
	0.0	26±4	29±3	124±2	124±4	26±4	29±3	124±2	124±4
SA	0.230	44±4	41±3	>2000	>2000				
AF	0.551	38±4	>5000	134±12	>2500				

The assay was performed with (+S9) and without (-S9) microsomal activation. Results are expressed as mean ± standard deviation of three replicate plates. FU: 5-fluorouracil, Do: doxorubicin, Cis: cisplatin, Car: carboplatin, Mt: methotrexate, Cp: ciclophosphamide, SA: sodium azide, AF: 2-aminofluorene. t: toxic for the *Salmonella* strain.

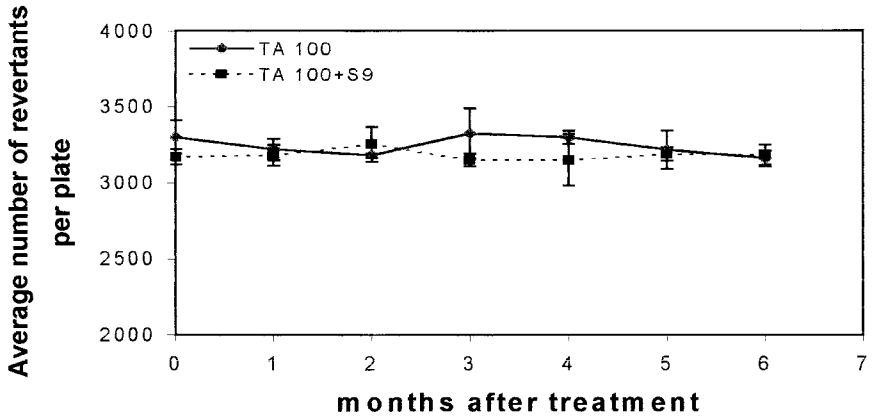

Figure 1. Persistence of mutagenicity generated by cyclophosphamide products after treatment. TA100 controls (not shown in the figure)140±15 revertants per plate.

The destruction of genotoxic wastes by high temperature incineration is a feasible and practical method of protection in spite of its technical difficulties and cost (ASHP 1985). However, frequently, wastes with antineoplastic drugs are mixed accidentally, intentionally or by mishandle, with health care wastes. Incorrect treatment can result not only useless but environmentaly harmful.

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