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Cytotoxicity and Genotoxicity of Untreated Hospital Effluents

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ABSTRACT

Untreated hospital effluent samples were tested for cytotoxic and genotoxicity in order to access whether they presented any serious health hazards. Results of umuC test suggested that the effluent from a large, general hospital could be considered weakly genotoxic. Comparatively, effluents from inpatient units presented higher frequency of genotoxicity than those coming from laboratory facilities. Although no cytotoxic activity was detected in laboratory effluent samples, cytotoxicity appeared to be an important problem of effluents from inpatient units.

Key words: Genotoxicity; cytotoxicity; untreated hospital effluents; water sources contamination

INTRODUCTION

The increase of human population is impacting the availability of drinkable water, both due to growing demands and because huge, uncoordinated, human concentrations have been throwing increasingly greater amounts of pollutants into water streams. The most severe problem is related with the disposal of non-treated, contaminated human dejects and toxic substances, generated by industrial activities and health service units, which are directly dispensed into the sewage, reaching sources of water supply.

Hospital effluents are known sources of several chemicals, remnants of medicine, disinfectants and antineoplastic drugs regarded as risky for humans and the environment (Giuliani et al.; 1996, Hartmann, 1998). These chemicals are potentially genotoxic, which is defined as the capacity to interfere with gene expression of cells. Besides this, residues associated with patient excretions may also contain several products with mutagenic activity (Dion and Bruce, 1983; Reddy et al., 1984; Reddy et al., 1985; Sousa et al., 1985; Fracasso et al., 1993; Choi et al., 1995).

One way to assess the genotoxicity of complex mixtures, as is the case of effluents, is to use short-term *in vitro* tests, such as bacterial bioassays (Gauthier et al., 1993). The umuC test, described by Oda et al. (1985), was developed to detect the presence of compounds presenting genotoxic properties. For a large number of environmental samples, it seems to be the most practical and least expensive test, being statistically equivalent to the Ames test (McDaniels et al., 1990).

The umuC test uses a genetically modified strain of *Salmonella typhimurium* (TA1535/pSK 1002), containing a multiple-copy plasmid, which contains a fusioned umuC::lacZ gene. This construct is comprised of a lacZ gene fusioned

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with the promoter and part of the umuC gene, which belongs to the operon umu that takes part in the SOS repair system in *E. coli* (Shinagawa et al., 1983; Oda et al., 1985; Whong et al.; 1986; MacDaniels et al., 1990). The induction of umuC gene expression shows a genotoxic activity and is defined by a β-galactosidase activity twice as high as that of the baseline (Pal et al., 1992). The selection marker for these bacteria is an ampicillin-resistance gene, also coded by psk 1002 plasmid. This strain has high permeability to chemicals and lacks a repair system by excision of nucleotides (uvrB), which increases its sensitivity after exposure to genotoxic, carcinogenic or mutagenic agents (Oda et al., 1985; Pal et al., 1992; Heil et al., 1996).

Giuliani et al. (1996) used the umuC test for the analysis of the polluting load generated by a hospital with 1400 beds in Zurich, Switzerland. The authors reported that considerable amounts of genotoxic substances were released into the environment through hospital effluents. Thus, these seem to present a potential environmental risk, even when diluted in other urban effluents (Leprat, 1999).

Although there are not many studies for hospital effluents, it is possible to question as to the danger they may represent for public health, since they are usually dispensed directly into the water resources without treatment. Therefore, from a genotoxic perspective, hospital effluents may cause an environmental impact, whose significance for human health is still largely unknown. In the present study, we evaluated the possible presence of cytotoxic (that is, cell direct toxicity) and genotoxic activities in the effluents of a typically large general hospital, in this case the *Hospital de Clínicas* (HCPA) *de Porto Alegre*, a big southern city of Brazil. The HCPA has about 725 beds and generates approximately 27,000 m³ of effluents a year. These effluents are dispensed untreated into the public sewage system that flows into Guaiba Lake, the main source of drinkable water for the city of Porto Alegre.

MATERIALS AND METHODS

Samples of untreated effluent from the HCPA were evaluated. The analyses were accomplished by 14 samplings comprised of effluents generated in two inpatient units (INP) and the hospital laboratory (LAB), making up to a total of 56 samples. Samples were evenly collected throughout one year at intervals of 26 days between them. Inpatient units and laboratory were chosen because these areas drained about 65% of the effluents generated by the hospital. The used procedure to carry out the umuC test was described by Oda et al. (1985), with modifications proposed by Hartmann (1998). The test was performed in both the presence and absence of a metabolic activation system (S9). Positive controls were 2-aminoanthracene and mitomicynC. The units of β-gal were determined by means of optical density measured at 600 and 420 nm and calculated according to the following formula:

$$U\beta gal = 1000 \frac{OD_{420}}{tv} \quad (1)$$

Where t is the incubation time (min), and v is the sample volume (mL).

The genotoxic activity, expressed as an induction factor (IF), is based on the ratio between the β-gal activity of the sample and that of the negative control, as expressed in the calculation below:

$$IF = \frac{\beta gal\ sample\ activity}{\beta gal\ control\ activity} \quad (2)$$

When IF = 1, the sample was considered negative for genotoxicity; otherwise, if IF ≥ 2, the sample was positive for genotoxicity. The criteria used for the interpretation of the results from the umuC test were as follows. Genotoxic samples are the ones that induced at least twice as much the induction as observed for the negative control; cytotoxic samples are the ones that presented a survival lower than 70% in the evaluation of cell viability (Oda et al., 1985; Hartmann, 1998 and Picada et al., 1997).

RESULTS AND DISCUSSION

Table 1 summarizes the results of the umuC test of samples collection from the HCPA and provides the mean values of β-galactosidase induction factors (IF) along with the cell survival rate (%S) for the line tested in every sample. The samples in which the induction factors were higher than or

equal to 2 (IF ≥ 2), with survival equal to or above 70%, were considered genotoxic. Cell survival values below 70% were classified as cytotoxicity effects (Vargas et al., 1993; Picada et al., 1997). According to the umuC test protocol (Oda et al., 1985), modified by Hartmann (1998), growth values up to 120 and 140%, compared to the negative control, in the absence and presence of the metabolic activation system (S9), respectively, were considered normal. According to the classification of Nakamura et al. (1987), the genotoxic responses for the effluents from the HCPA (table 1) were, in general weakly positive (+), showing only a duplication of enzymatic induction when compared to the baseline.

The results for the effluents generated in laboratory areas revealed that though it had a positive response in one of the samples without S9 (sample 11), such response was weak. For the effluents from inpatient areas, only sample 8 in the absence of S9 showed high induction levels (IF=6.51) for a 48% survival. When sample 8 was subjected to this test again, there was a higher survival rate (62%), and it was classified as intermediate genotoxic (++), i.e., it presented a three times increased gene expression when compared to the baseline (Nakamura et al., 1987), and, although S% was slightly lower than 70%, the observed IF value of 4.18 indicated the presence of a positive genotoxic response.

Table 1 - Values of the induction factor of β-galactosidase (IF) in *Salmonella typhimurium* (TA1535/pSK 1002) in effluent samples from the HCPA inpatient and laboratory areas in the absence (without S9) and presence (with S9) of metabolic activation.

Sample	Inpatient				Laboratory			
	without S9		with S9		without S9		with S9	
	IF [a]	%S [b]	IF	%S	IF	%S	IF	%S
1	2.17	63	1.13	87	0.91	147	0.55	98
2	2.32	72	1.87	72	1.22	109	0.96	108
3	1.18	95	1.35	100	1.08	111	1.12	96
4	1.59	71	1.40	64	1.10	102	1.05	101
5	2.27	75	3.60	56	1.24	90	0.92	110
6	1.73	64	2.38	62	1.02	114	0.92	107
7	1.02	97	1.24	92	1.01	108	0.94	108
8	6.51	48	5.41	50	1.02	97	0.59	126
9	2.99	64	4.97	54	1.19	104	1.24	96
10	1.36	89	1.70	76	1.06	106	1.37	91
11	1.26	113	2.62	69	2.22	87	0.97	106
12	2.94	58	6.54	49	0.85	113	1.04	106
13	1.26	95	1.38	92	1.15	106	1.23	109
14	1.13	103	1.04	109	1.10	90	0.70	111

[a] The induction factor (IF) is defined as units of β-galactosidase of sample per units β-galactosidase of negative control.
[b] The cell survival rate was calculated relative to the negative control as a function of absorbance (600nm).

When the tests for inpatient effluent samples 5 and 12 were repeated, a weak genotoxic activity was confirmed, with IF=2.08 for 88% S%, and IF=2.40 for 76% S%. Equally weak was the response presented in sample 2, also collected from inpatient areas, in the absence of metabolization.

In Table 1, five genotoxic samples (18%) and five cytotoxic samples (18%) were present in the absence of S9. In the presence of metabolization, seven cytotoxic samples (25%) and no genotoxic sample were obtained. Results also showed a mild predominance of cell viability reduction in the presence of S9 (Table 2). For effluent samples from inpatient areas, 50% of the samples presented cytotoxicity in the presence of S9 and 36% in its absence. No effluent sample from laboratory areas

had a cytotoxic effect either in the presence, or absence of S9. According to Legault et al., (1996), the toxicity reduction in the presence of S9 might be ascribed to detoxification processes or non-specific bindings between toxic molecules and S9 proteins.

In the global analysis of the data in this study, it was found that for the effluents from both inpatient and laboratory areas (Tables 1 and 2), there was a loss of genotoxic activity in all samples with induction in the absence of metabolization, after metabolic activation; in other words, there was a response with induction of enzymatic activity only in the absence of S9. The disappearance of genotoxic activity in the presence of metabolization could be correlated to the genotoxic activity of the effluent caused by direct mutagenic agents inactivated by the metabolization system through detoxification catalyzed by these enzymes. However, the exact mechanism of genotoxicity reduction in the presence of S9 remained unclear. Metabolization may provide even more toxic characteristics to the analyzed samples, probably due to the high activity of metabolites generated by available substances, which led, in some cases the absence of genotoxiciy. Besides, the high toxicity presented by these samples might point out to a possible contamination by biologically active substances, such as antibiotics and disinfectants (Saxena, 1984; Houk, 1992; Vargas et al., 1993; Giuliani et al., 1996; White et al., 1996). These substances are widely used at the HCPA, and their ultimate destination is the effluents.

Table 2 - Frequencies of responses to the umuC test in effluent samples from inpatient areas (INP) and laboratory facilities (LAB) of the HCPA in the absence (without S9) and presence (with S9) of metabolic activation.

Response	**Local**	**without S9**		**with S9**	
		F a	f (%) b	F	f (%)
Genotoxic	INP	4	29	0	0
	LAB	1	7	0	0
	total	5	18	0	0
Cytotoxic	INP	5	36	7	50
	LAB	0	0	0	0
	total	5	18	7	25

[a] Number of samples with cytotoxic or genotoxic response.
[b] Relative frequency of samples with cytotoxic or genotoxic response relatively to the total of samples from each area.

Of the total of analyzed effluent samples from the HCPA, not more than 18% without S9 had values indicating genotoxic activity. These data seemed to be in agreement with Giuliani et al. (1996), who observed, in an evaluation carried out in a hospital with 1,400 beds, using the umuC test in the absence of metabolization, a rate of genotoxic samples (with IF $\geq$ 2) of 13%. These authors, analyzing drugs widely used in hospitals, claimed that the chemicals related to genotoxic effects, which were not associated to cytotoxicity, were probably the antineoplastic drugs. The associated cytotoxic effect would probably be resultant from the presence of antibiotics and/or disinfectants. As the handling and disposal of antineoplastic drugs is strictly regulated, the main route of access of these substances to the effluent may be through the excretions of patients under treatment with these drugs.

In eight cases, an occurrence of effect caused by the effluent toxicity was observed (Table 1 – samples from inpatient areas 1 and 9 without S9, and samples 5, 6, 8, 9, 11 and 12 with S9). Although the observed induction rates were high, compared to the baseline, there was a low cell viability, which primarily characterizes a cytotoxic effect. The 25% reduction in cell viability in samples could have caused an overestimation of genotoxic activity. If this was the case, it would be suitable to apply other genotoxicity tests such as the SOS chromotest, which used controls of cell viability by monitoring the alkaline phosphatase activity (Quillardet and Hofnung, 1988; Picada et al., 1997; White et al., 1998).

Low-survival samples were observed only in the effluents from inpatient areas. On average, for these sites, the survival values without metabolization were slightly higher (79.1%) than those observed with metabolization (73.8%). The

effluents generated in laboratory areas had mean survival values around 105% (with and without S9). These data revealed the presence of a higher toxicity in the effluents generated in inpatient areas.

Although pH values were not measured, the cytotoxic effect presented by the effluent could also be ascribed to this variable (Vargas, 1993), as cell death could rise as result of a primary damage to DNA, with breakdowns and bridges, or as the result of a more generalized toxic effect in the cell, similar to that occurring in the presence of detergent, which promoted the dissolution of cell membranes. Giuliani et al. (1996), analyzing substances such as disinfectants and antibiotics, concluded that at high concentrations, disinfectants present significant cytotoxic effects, which were able to promote the induction of β-gal. Antibiotics, such as chloranphenicol, tetracycline and erythromycin, in high concentrations, were also able to induce β-gal.

Of the total of 56 samples analyzed in this study, 32 (57.1%) did not present any genotoxic or cytotoxic effect, either in the presence of S9 or in its absence. Taking into account the absence of S9 only, 18 samples (64.3%) were observed to have these characteristics.

For a comparison between the results from Giuliani (1994) and Giuliani et al. (1996), only the results obtained in the absence of metabolization must be considered. These authors observed that 96% of their samples had not presented cytotoxic effect and only 4% had presented a combination of cytotoxic and genotoxic effects. In this study, taking into account the samples without S9 only, the rates were 82.1% and 3.6%, respectively. To support their data, the authors established comparisons between the values found in their study and those found by Gartiser et al. (1994), who conducted analyses of effluents from a similarly sized hospital, using the microsome/ *Salmonella* test (AMES test). Giuliani et al. (1996) concluded that there was a similarity in the effluents from both hospitals. The data presented provided evidence that there was a similarity between the cytotoxic and the genotoxic activities for the effluents from both hospitals (Gartiser et al., 1994; Giuliani et al., 1996) and those generated at the HCPA, which had no effluent treatment facilities.

As hospital sewages are complex mixtures, the umuC test must be considered as a relevant indication for potential risks to human health and the aquatic environment. However, according to Hartmann (1998), the positive responses for genotoxicity resulting from this test in hospital samples should be analyzed regarding the present fluorokinolone concentrations, as these drugs might interfere with the induction of the SOS response, monitored by this test. Hence, the samples should be further tested by another method that did not use the SOS response as an indicator. Thus, the possible risks for human health and environment contamination would be more accurately ascertained.

CONCLUSIONS

Overall, the effluent from the Hospital de Clínicas de Porto Alegre could be considered weakly genotoxic. Comparatively, the effluents from inpatient areas presented a higher frequency of genotoxic samples as compared to those from laboratory areas. Although no cytotoxic activity was detected in the effluent samples from the laboratory, in the samples from inpatient units, toxicity seemed to be a great problem, since it was present in 50% of the samples from these areas. Therefore, in future studies, these effluents should be tested through other methods, such as the microsome/*Salmonella* test, for instance, in order to ascertain potential mutagenic effects and the type of induced mutations, in addition to an assessment of toxicity through traditional ecotoxicological tests.

Based on the results from the present study, it could be concluded that hospital effluents, especially those generated in inpatient areas, carried substances with genotoxic and cytotoxic activity. Thus, they could pose a potential risk to public health through the circulation of agents in the environment, animals and people and should be treated before discharge.

RESUMO

Amostras de efluentes hospitalares não tratados foram testadas para determinação de efeitos citotóxicos e genotóxicos para verificar a possibilidade destes apresentarem riscos à saúde. Resultados do ensaio umuC sugeriram que o efluente de um grande hospital de clínicas gerais

poderia ser considerado fracamente genotóxico. Comparativamente, efluentes de unidades de internação apresentaram freqüência maior de genotixicidade, quando comparados com efluentes de setores laboratoriais. Embora nenhuma atividade citotóxica tenha sido detectada em amostras dos efluentes de unidades laboratoriais, para unidades de internação esta atividade parece ser um problema importante.

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