Chemical Degradation of Wastes of Antineoplastic Agents Amsacrine, Azathioprine, Asparaginase and Thiotepa

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As a part of a program devoted to the destruction of antineoplastic agents, three chemical methods readily available in the hospital environment, viz. oxidation with sodium hypochlorite (NaClO, 5%), hydrogen peroxide (H₂O₂, 30%), and Fenton reagent (FeCl₂·2H₂O; 0.3 g in 10 ml H₂O₂, 30%), were tested for the degradation of four anticancer drugs: Amsacrine, Azathioprine, Asparaginase and Thiotepa. The efficiency of the degradation was monitored by high-performance liquid chromatography. The mutagenicity of the degradation residues were tested by Ames test using tester strains Salmonella typhimurium TA 97a, TA 98, TA 100 and TA 102 with and without an exogenous metabolic activation system. Using sodium hypochlorite, 98.5% of Amsacrine, 99.0% of Azathioprine, 99.5% of Asparaginase and 98.7% of Thiotepa were destroyed after 1 hr. The hydrogen peroxide treatment destroyed 99% of Asparaginase and 98.7% of Thiotepa in 1 hr. However, this procedure was not efficient for the treatment of Amsacrine (28% after 16 hr) and of Azathioprine (53% degradation in 4 hr). The action of Fenton reagent resulted in the destruction of 98% of Amsacrine, and 99.5% of Azathioprine, 98.5% of Asparaginase and 98.7% of Thiotepa in 1 hr. In all cases where a high degree of degradation was achieved, the residues obtained were non mutagenic.

INTRODUCTION

Several cytostatic drugs have been shown to be mutagenic, teratogenic and carcinogenic in experimental systems (Sorsa et al., 1985). A number of cytostatic drugs have been classified by the International Agency for Research on Cancer (IARC, 1994) as human carcinogens (group 1), probable human carcinogens (group 2A) or possible human carcinogens (group 2B). Among the four compounds considered in this study Thiotepa has been classified in group 1 while the others have not yet been evaluated by the IARC working group. Thus, hospital personnel handling these drugs may have an occupational risk. Inhalation and skin absorption are the main routes of contamination in unprotected individuals. Inhalation may occur by formation of aerosols during drug preparations. Skin absorption occurs by direct contact with the compounds, after spillage and during the handling of biological fluids of patients undergoing chemotherapy (see De Méo et al. (1995) and references therein). Numerous references reporting the risks of exposure of pharmacy, nursing and other hospital personnel to these mutagenic drugs can be found in our previous paper (Hansel et al., 1997). Possible adverse health effects caused by the handling of these drugs have been reviewed (Rousselin and Stucker, 1990). Hospital staff or family members handling the excreta from patients treated with antineoplastic drugs or equipment contaminated by these excreta, such as urinals and chamber pots, may be more heavily exposed to the drugs or their metabolites than trained hospital nurses or pharmacists (Guinee et al., 1991; Cass and Musgrave, 1992; Sessink et al., 1992).

The handling of these drugs, in pure form or in solution, generates residual amounts of cytotoxic solutions, which should not be discarded in the domestic sewage system without prior inactivation. In 1979, a program for the development of chemical methods for the treatment of wastes contaminated with chemical carcinogens was initiated by the International Agency for Research on Cancer, with the support of the Office...
of Safety of the National Institutes of Health (IARC, 1979). In 1985, antineoplastic drugs were considered, in this program (Castegnaro et al., 1985). Since then, several publications have been issued (Barek et al., 1987; Monteith et al., 1987; Lunn et al., 1989; Benvenuto et al., 1993; Allwood and Wright, 1993) describing the destruction of antineoplastic drugs using acids or strong oxidizing agents, such as potassium permanganate, which are not considered acceptable by hospital staff. A new program was thus initiated by IARC with the support of the French Ministry of the Environment and of the National Institutes of Health to systematically investigate the efficiency of 5% sodium hypochlorite and 30% hydrogen peroxide in the degradation of 36 antineoplastic agents. (These two reagents were indicated acceptable in a survey conducted among the nurses, pharmacists and members of the Hygiene and Safety Committee of the Hospitals of Montpellier (France)). Five laboratories from France, the Czech Republic and the United States were involved in the testing of the efficiency of degradation of these compounds and of the residues for mutagenic activity. So far, the results of destruction of cyclophosphamide, ifosfamide and melphalan (Hansel et al., 1997) and of six anthracyclines (Castegnaro et al. (1997) submitted for publication) using sodium hypochlorite, hydrogen peroxide and Fenton reagent were reported. This paper is devoted to the degradation of residual pharmaceutical solutions contaminated by Amsacrine, Azathioprine, Asparaginase and Thiotepa. As in the previous IARC investigations, the effectiveness of the degradation or inactivation methods was determined by HPLC for monitoring the disappearance of the parent compound. Mutagenicity was then chosen as an indicator of potential toxic or genotoxic effects of the degradation products.

Amsacrine is an acridine-like intercalating agent [see Fig. 1(a)] currently used as a chemotherapeutic agent for the treatment of acute leukaemia and solid tumours. Therapeutic activity was seen in Hodgkin’s disease, hepatoma and epidermoid carcinoma of the oesophagus. Azathioprine is an immunosuppressant and antineoplastic agent [see Fig. 1(b)] with similar action to those of mercaptopurine to which it is slowly converted in the body. Its main use involves the immunosuppressant properties. Asparaginase is a therapeutic enzyme mainly used in combination with other agents in the treatment of acute lymphatic leukaemia. Thiotepa is a polyfunctional alkylating agent [see Fig. 1(c)] mainly used in the treatment of adenocarcinoma of the breast and of the ovary, and of superficial papillary carcinoma of the bladder. It is also used for controlling intracavitary effusions secondary to diffuse or localised neoplastic diseases of various serosal cavities and has been found effective against lymphosarcoma and Hodgkin’s disease.

The three above mentioned methods, based on treatment with sodium hypochlorite, hydrogen peroxide and Fenton reagent were investigated on: (1) aqueous solutions of Amsacrine formulation (0.15 mg/ml) in 5% D-glucose; (2) aqueous solutions of Azathioprine formulation (2 mg/ml) in 5% D-glucose; (3) aqueous solutions of Asparaginase formulation containing 200 U of Asparaginase, 1.6 mg of mannitol and 45 mg of D-glucose per 1 ml and (4) aqueous solutions of the Thiotepa formulation (0.016 mg/ml) in 5% D-glucose.

MATERIALS AND METHODS

Warning
Several antineoplastic agents are carcinogenic to humans (Thiotepa has been classified in group I by IARC) and must be handled with great care. Appropriate precautions must be taken and protective clothing such as latex gloves, eye goggles and laboratory coat must be worn.

Reagents
Reference compounds. Amsacrine (as a solution in anhydrous N,N-dimethylacetamide containing 50 mg of Amsacrine per 1 ml) was obtained from National Cancer Institute (Bethesda, Maryland, U.S.A.). The reconstitution solution containing 5 mg ml of Amsacrine in 10% v/v of N,N-dimethylacetamide and 0.318
mol/l l-lactic acid was prepared according to a prescribed procedure, i.e. by injecting 1.5 ml of the supplied solution of Amsacrine (50 mg/ml in anhydrous N,N-dimethylacetamide) into the 13.5 ml of supplied sterile diluent (0.0353 mol/l l-lactic acid). Stock solution of Amsacrine (0.15 mg/ml of Amsacrine in 5% d-glucose) was prepared by diluting 3 ml of reconstituted solution with 5% aqueous solution of d-glucose to 100 ml. The stock solution was kept at 4°C. All destructions were carried out within 4 hours after the preparation of the stock solution.

Azathioprine formulation (as the disodium salt) was obtained from Burroughs Wellcome Co. (Research Triangle Park, NC, U.S.A.). Stock solution (2 mg/ml of Azathioprine in 5% d-glucose) was prepared by dissolving 100 mg of Azathioprine in 50 ml of 5% solution of d-glucose in deionised water. The stock solution was kept at 4°C. All destructions were carried out within 24 hours after the preparation of the stock solution.

Asparaginase (as a white powder containing 10,000 U of Asparaginase and 80 mg of mannitol per vial) was obtained from Merck, Sharp Dome (West Point, PA, U.S.A.). Asparaginase was dissolved by injecting 5 ml of deionised water into the vial, giving rise to solution containing 2000 U/ml and 16 mg of mannitol per 1 ml. Stock solution containing 2001 U/ml of Asparaginase, 1.6 mg of mannitol and 45 mg of d-glucose per 1 ml was prepared by exact 1:10 dilution of the initial solution with solution of 5% d-glucose in deionised water. All destructions were carried out within 4 hours after the preparation of the stock solution.

Thiotepa as a lyophilised powder was supplied by Lederle Laboratories Ltd. Stock solution containing 0.06 mg/ml in 5% d-glucose was prepared by dissolving 3 mg of Thiotepa in 50 ml of 5% solution of glucose in deionised water. All destructions were carried out within 24 hours after the preparation of the stock solution.

Reagents for degradation. Sodium hypochlorite (5% aqueous solution) and hydrogen peroxide (30% aqueous solution) were obtained as commercial products. FeCl₂·2H₂O was purchased from Merck (Darmstadt, Germany). Fenton reagent (0.3 g of FeCl₂·2H₂O per 10 ml of 30% H₂O₂) was prepared in situ. Solutions of sodium hypochlorite and hydrogen peroxide were stored in well-filled airtight containers at a temperature not exceeding 20°C and were protected from light. Their activity was tested prior to use as described by Castegnaro et al. (1991) for the sodium hypochlorite and in Pharmacopée Française (1992) for hydrogen peroxide.

Other reagents. Methanol, acetonitrile, triethylamine phosphate, potassium dihydrogenphosphate, glacial acetic acid, sodium hydroxide, disodium salt of ethylenediamine tetraacetic acid (used in HPLC monitoring of the efficiency of the degradation) were purchased from Merck (Darmstadt, Germany).

Apparatus and HPLC conditions

HPLC system. LKB 2150 HPLC pump (Bromma, Sweden) equipped with LKB 2151 Variable Wavelength Monitor, LCI 30 injection valve with a 20 µl loop (Laboratorni přístroje, Prague, Czech Republic), and PC integrator INCOM (Labio, Prague, Czech Republic) supported with Xerius DX 386 33 MHz computer (CS21, Prague, Czech Republic).

HPLC conditions. Amsacrine: The HPLC procedure was a modified version of the technique of Jurlina and Paxton (1983). Briefly, the analyses were performed on a reversed-phase C18 analytical columns (Tessek, Prague, Czech Republic, particle size 5 µm; 150 mm long x 3 mm i.d.) using isocratic conditions. Degradation with sodium hypochlorite and hydrogen peroxide was monitored using the mobile phase acetone-water-aqueous 1M triethyamine phosphate (60+40+1). The pH of this mobile phase was 3.5 and the flow rate was maintained at 0.5 ml/min. Degradation with Fenton reagent was monitored using mobile phase methanol—0.025M KH₂PO₄—glacial acetic acid (20+79.5+0.5). The pH of this mobile phase was 4.5 and the flow rate was maintained at 0.4 ml/min. UV detection at 270 nm was used throughout. Under these conditions, Amsacrine had a retention time of 5.5 minutes.

Azathioprine: The HPLC procedure was a modified version of the method of Fell et al. (1979). Briefly, the analyses were performed on a reversed-phase C18 analytical columns (Tessek, Prague, Czech Republic, particle size 5 µm; 150 mm long x 3 mm i.d.) using isocratic conditions. Degradation with sodium hypochlorite and hydrogen peroxide was monitored using mobile phase methanol-aqueous 0.025M KH₂PO₄—glacial acetic acid (200+795+5) adjusted to pH 4.5 by 10 M KOH. Degradation with Fenton reagent was monitored using mobile phase MeOH-aqueous 0.025M KH₂PO₄—glacial acetic acid (10+40+3). The pH of this mobile phase was 2.8 to prevent precipitation of Fe(III) oxides after degradation with Fenton reagent. The flow rate was maintained at 0.3 ml/min and UV photometric detection at 281 nm was used throughout. Under these conditions, Azathioprine had a retention time of 24.5 minutes.

Asparaginase: Previous work for the determination of Asparaginase made use of the reversed-phase high-performance liquid chromatography (Kalghatgi and Horvath, 1987) and non-porous crosslinked polystyrene particles having a mean particle diameter of 3 µm (Maa and Horvath, 1988) coupled with UV detection at 210 nm was used in both cases. Recently, hydroxyethyl methacrylate-based (HEMA) sorbents were successfully used for high-performance liquid chromatography (Čoupek and Vinš, 1994). We therefore used this sorbent for HPLC monitoring of the efficiency of degradation of Asparaginase. All the analyses were performed on a HEMA—BIO 1000 analytical column, particle size 10 µm, 250 mm long x 4 mm i.d. (Tessek, Prague, Czech Republic) using iso-
cratic conditions with 0.1M KH₂PO₄ solution in deionised water adjusted with 2 M sodium hydroxide (NaOH) to pH 6.9 as a mobile phase. The flow rate of 0.8 ml/min and UV photometric detection at 210 nm was used throughout. Under these conditions, Asparaginase had a retention time of 2.1 minutes.

**Thiopeta: HPLC procedure** was a modified version of the technique of Tinsley et al. (1889). Briefly, the analyses were performed on a reversed-phase C18 analytical columns (Tessek, Prague, Czech Republic, particle size 5 μm; 150 mm long x 3 mm i.d.) using isocratic conditions with mobile phase acetonitrile-water (20:80). The flow rate was maintained at 0.4 ml/min and UV photometric detection at 200 nm was used throughout. Under these conditions, Thiopeta had a retention time of 11 minutes.

All HPLC assays were performed at ambient temperature.

**Mutation assay**

Mammalian liver S9 preparation: Sprague–Dawley rats weighing approximately 200 g were injected with Aroclor 1254 five days before sacrifice. Post-mitochondrial supernatant from homogenised rats livers (S9) was prepared as described by Maron and Ames (1983) and stored at −80°C. The protein content of the S9 fraction determined according to Lowry et al. (1951) was 31.6 mg/ml.

The plate incorporation technique of Salmonella Mammalian Microsome Mutagenicity Assay was performed essentially as recommended by Maron and Ames (1983) with the modification of De Méo et al. (1996). The Salmonella Typhimurium tester strains TA 97a, TA 98, TA 100, TA 102 were used in the presence or absence of a metabolic activation system S9 mix formed essentially as recommended by Maron and Ames (1983).

Volumes of 0.1 ml of S9 mix, various amounts of the tested samples not exceeding 10 ml and 0.1 ml of the overnight culture were incubated at 37°C for 60 min with rapid shaking. Two ml of melted top agar were added to the tubes. The mixtures were poured onto VB minimal salt agar plates. For each experiment the controls included:

- 10 μl of ultrapure water to determine the frequency of spontaneous revertants.
- A positive control for each tester strain:
  - 20 ng of 2-methoxy-6-chloro-9-(2-chloroethyl) aminopropylaminoacridine (ICR191) for tester strain TA 97a.
  - 20 ng of 2,4,7-trinitro-9-fluorenone (2,4,7-TNFone) for tester strain TA 98.
  - 0.5 μg of sodium azide for tester strain TA 100.
  - 20 ng of mitomycin C for tester strain TA 102.
- 0.5 μg of benzo[a]pyrene with tester strains TA 97a, TA 98, TA 100 and TA 102 for quality control of the S9 mix.

After a 48 hr incubation period, plates were counted using an automatic laser colony counter with a bacterial enumeration programme (Spiral system instruments INC., Bethesda, MD, U.S.A.).

**Degradation procedures**

**Degradation with sodium hypochlorite.** The stock solutions of the antineoplastic drugs (1 ml) were mixed with equal volume (1 ml) of aqueous sodium hypochlorite solution (5%) and stirred for 1, 2, or 16 hr, respectively, on a magnetic stirrer. Afterwards, conc. HCl was added dropwise until pH 3–4 was reached (checked with pH paper) while flushing out the chlorine with nitrogen. Then, the solution was bubbled with nitrogen for further 10 minutes to remove all remaining traces of chlorine.

**Degradation with hydrogen peroxide.** The stock solutions of the antineoplastic drugs (1 ml) were mixed with equal volume (1 ml) of aqueous solution of hydrogen peroxide (30%) and stirred for 1, 4, or 16 hr, respectively, on a magnetic stirrer. Afterwards, 1.2 g of Na₂S₂O₃ was added whilst cooling in an ice bath.

**Degradation by Fenton reagent.** The stock solutions of the antineoplastic drugs (1 ml) were mixed with equal volume (1 ml) of aqueous solution of hydrochloric acid (2 mol·l⁻¹) and 0.3 g of FeCl₃·2H₂O was added. Afterwards, 10 ml of aqueous solution of hydrogen peroxide (30%) were slowly added whilst stirring. Then the solution was set aside for 30 min to cool.

**Caution:** the degradation must be performed in a glass Erlenmeyer flask at least 5 times larger than the final volume of solution and reagent used and the reaction vessel must be kept in an ice bath to avoid problems of an exothermic reaction.

All degradations were also performed on blank solutions (without antineoplastic agent) as controls for HPLC and mutagenicity testing.

**Analytical procedures**

Volumes of 20 μl of the solution after destruction with sodium hypochlorite or hydrogen peroxide were injected directly into HPLC. Afterwards, aliquots of the solution, after destruction, were spiked with various volumes of the original stock solution and 20 μl of the resulting solution were injected into HPLC.

In the case of degradation of Amsacrine by Fenton reagent, it was necessary to add 0.4 g of the disodium salt of ethylenediaminetetraacetic acid to the solution after destruction to prevent precipitation of iron oxides.

In the case of Asparaginase, prior to HPLC analysis, the pH of the solution after degradation was adjusted to 7 with 2 M NaOH (checked with universal pH paper). When Fenton reagent was used, solid ferric hydroxide precipitated at this pH and was removed by filtration through paper filter.
RESULTS AND DISCUSSION

HPLC analysis

Under the above mentioned analytical conditions, the limits of detection based on a 3 fold signal to noise ratio were: Amsacrine, 0.1 μg/ml; Asparaginase, 0.51 U/ml; Thiotepa, 0.5 μg/ml and Azathioprine, 1 μg/ml.

Degradation with sodium hypochochlorite HPLC assays

Amsacrine: Comparison of the peak of the degraded sample and the corresponding spiked sample demonstrate that at least 98.5% of Amsacrine was destroyed after 1 hr, 99.1% after 2 hr and 99.3% after 16 hr [Fig. 2(a)]. The height and the area of the peak corresponding to the spiked solution slightly decrease with time elapsed from the addition of the spike to the injection into HPLC. It probably means, that Amsacrine decomposes even after acidification of the solution of NaCIO either because of traces of chlorine present or because of acidic conditions.

Azathioprine: Comparison of the peak area of the degraded sample and spiked sample showed that at least 99.0% of Azathioprine was destroyed after 1 hr, 99.2% after 2 hr and 99.5% after 16 hr. (The chromatograms after 16 hr is shown in Fig. 2(b)).

Asparaginase: No HPLC peak corresponding to Asparaginase could be detected in the solution after 1 hr degradation with sodium hypochochlorite [see Fig. 2(c)]. In the solution after the standard addition of Asparaginase corresponding to 0.5% of the original amount of this substance this peak could be detected [see Fig. 2(c)]. The limit of detection of Asparaginase under the analytical conditions used corresponds to 0.5% of the amount of Asparaginase originally present in the solution prior to degradation. Therefore, it can be concluded that the efficiency of the degradation is at least 99.5%. (The real value of the degree of degradation is probably even higher, the above given value being affected by the limit of detection of our analytical method). Since Asparaginase is unstable and decomposes under acidic conditions (Allwood and Wright, 1993), it was necessary to adjust the pH to 7 after the degradation, otherwise no peak of Asparaginase could be detected by the standard addition.

Thiotepa: Thiotepa is quickly decomposed under acidic conditions (Allwood and Wright, 1993). It is therefore not possible to use the standard addition method for its quantitation on the acidified degradation mixtures. After adjusting to pH 7 we were able to detect the standard addition corresponding to 5% of the originally present amount of Thiotepa while the peak of this substance in the solution after destruction was not detectable [see Fig. 2(d)]. Thus it can be concluded that at least 95% of Thiotepa was degraded by this procedure. However, even under these conditions, the height of the peak of Thiotepa decreased by about 20% during 20 minutes after the standard addition. Therefore, we can assume that the peak corresponding to the standard addition would be higher without the observed decomposition of Thiotepa. It means that the efficiency of the method is probably even higher than 95%. According to the limit of detection of the Thiotepa specified above, the degradation is probably >98.7%.

![Fig. 2. Chromatograms of degradation with sodium hypochochlorite.](https://academic.oup.com/annweh/article-abstract/42/4/259/149172)
Degradation with hydrogen peroxide

**Amsacrine:** From the comparison of the area of the treated and spiked solutions, it was calculated that only 15% of Amsacrine was destroyed after 4 hr and 28% after 16 hr. Therefore, the method was not efficient for practical purposes and mutation assays were not performed.

**Azathioprine:** From the comparison of the area of the peaks of the treated and spiked solutions it was determined that only 53% of Azathioprine was destroyed after 4 hr and 65% after 16 hr. Therefore, the method is not efficient enough for practical purposes and mutation assays were not performed.

**Asparaginase:** No peak of Asparaginase at retention time 2.1 min could be detected in the solution after 1 hr degradation with hydrogen peroxide [see Fig. 3(a)]. In the solution after the standard addition of Asparaginase corresponding to 1% of the original amount of this substance this peak could be detected [see Fig. 3(a)]. It can be calculated from the signal to noise ratio at the retention time 2.1 min that the limit of detection of Asparaginase under these conditions corresponds to 1% of the amount of Asparaginase originally present in the solution prior to degradation. Therefore, it can be concluded that the efficiency of the degradation is at least 99.0%. (The real value of the degree of degradation is probably even higher, the above given value being affected by the limit of detection of our analytical method). It was again necessary to adjust the pH to 7 after the degradation for the above explained reasons.

**Thiotepa:** Again, no peak of Thiotepa can be detected in the solution after destruction without the adjustment of pH to 7. However, the peak of Thiotepa after the standard addition corresponding to 50% of the originally present amount of Thiotepa could not either be detected. Possible explanation is again the lack of stability of Thiotepa in the solution after destruction. When the pH of the solution after destruction was adjusted to 7, we were able to detect the standard addition corresponding to 10% of the originally present amount of Thiotepa [see Fig. 3(b)]. Because the peak of Thiotepa in the solution after destruction was not detectable and in view of the limit of detectability of Thiotepa it was concluded that at least 98.7% was degraded by this procedure.

Degradation by Fenton reagent. **Amsacrine:** From the comparison of the area of the peak of the solution after destruction and after addition of the spike it was calculated that ca 98% of the Amsacrine was destroyed after 30 minutes and 99.5% after 16 hr [see Fig. 4(a)].

**Azathioprine:** No peak at retention time corresponding to Azathioprine. The spike corresponding to 0.5% of the original amounts resulted in a peak easily discernible from the noise [see Fig. 4(b)]. Therefore, it can be concluded that at least 99.5% of the originally present Azathioprine was destroyed.

**Asparaginase:** No peak was detectable at retention time of Asparaginase after degradation with Fenton reagent [see Fig. 4(c)]. In the solution after the standard addition of Asparaginase corresponding to 2% of the original amount of this substance this peak could be detected [see Fig. 4(c)]. It was therefore calculated from the signal to noise ratio at the retention time that the limit of detection of Asparaginase under these conditions corresponds to 2% of the amount of Asparaginase originally present in the solution prior to degradation. Therefore, it can be concluded that the efficiency of the degradation is at least 98%. (The real value of the degree of degradation is probably even higher, the above given value being affected by the limit of detection of our analytical method). It was again necessary to adjust the pH to 7 after the degradation for the above explained reasons. Although the adsorption of Asparaginase on freshly precipitated ferric hydroxide cannot be completely ruled out, the results of mutagenicity testing (see further) confirm the absence of Asparaginase in the solution after destruction.

**Thiotepa:** No peak of Thiotepa was detectable in the solution after destruction at retention time corresponding to Thiotepa [see Fig. 4(d)]. The standard addition corresponding to 50% of the originally present amount of the substance does not give any peak, either. As in the previous cases, instability of Thiotepa in the solution after destruction is a possible explanation. The method is probably very efficient. However, the efficiency cannot be checked by the standard addition method. The pH of the solution after destruction cannot be adjusted to 7 because of iron oxides precipitation.
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Fig. 4. Chromatograms of degradation with the Fenton reagent. [4a] Amsacrine solution degraded for 16 h before (1) and after (2) addition of standard solution of Amsacrine corresponding to 1% of the originally present amount; [4b] degraded Azathioprine solution before (1) and after (2) addition of standard solution of Azathioprine corresponding to 0.5% of the originally present amount. [4c] Asparaginase solution degraded for 1 h before (1) and after (2) addition of standard solution of Asparaginase corresponding to 2% of the originally present amount. [4d] Thiotepa solution degraded for 1 h before (1) and after (2) addition of standard solution of Thiotepa corresponding to 10% of the originally present amount. An arrow indicates the position of the peak of the tested substance.

Mutagenicity test performance

For each compound an initial reaction time of one hour was adopted to measure the efficiency of the degradation. When incomplete destruction was noticed the reaction time was lengthened. When complete degradation was achieved, samples were prepared for mutagenicity testing by the removal of the oxidant using addition of solid sodium bisulphite and neutralisation with concentrated hydrochloric acid. These reactions were performed on ice to avoid an exothermic reaction.

Throughout this study, the background of spontaneous revertants/plate were: (i) TA 97a (–S9, 189 ± 28; + S9, 190 ± 24), (ii) TA 98 (–S9, 26 ± 8; + S9, 33 ± 7), (iii) TA 100 (–S9, 122 ± 19; + S9, 123 ± 20), (iv) TA 102 (–S9, 337 ± 37; + S9, 364 ± 44).

The response to the positive controls were: TA 97a (20 ng ICR 191) 2625 ± 304 revertants per plate; TA 98 (20 ng 2,4,7-TNFone) 725 ± 143 revertants per plate; TA 100 (0.5 μg sodium azide) 564 ± 63 revertants per plate; TA 102 (20 ng mitomycine C) 3315 ± 321 revertants per plate. The positive controls for S9 mix included (TA 97 + 0.5 μg benzo[a]pyrene) 1170 ± 241 revertants per plate, (TA 98 + 0.5 μg benzo[a]pyrene) 560 ± 67 revertants per plate, (TA 100 + 0.5 μg benzo[a]pyrene) 1404 ± 154 revertants per plate, (TA 102 + 0.5 μg benzo[a]pyrene) 744 ± 91 revertants per plate.

Mutagenicity testing of the residues

All residual solutions in which degradation was greater than 98% were tested for mutagenic activity under the above described conditions. None of them was found mutagenic. This is also valid for residual solutions after degradation of Thiotepa with sodium hypochlorite, hydrogen peroxide and Fenton reagent, where it was not possible to ascertain the chemical efficiency of the destruction after the addition of the spike.

CONCLUSIONS

The degradation of Amsacrine, Azathioprine, Asparaginase and Thiotepa with sodium hypochlorite is very efficient, easy to perform and uses reagents readily available in the hospital environment. This technique can be used to degrade expired drug solutions, excess of solutions prepared for administration to patients and to clean up spills, urinals, etc. Thus, the exposure of hospital personnel to the hazards of this carcinogenic drug can be reduced.

The degradation of Amsacrine, Azathioprine, Asparaginase and Thiotepa with Fenton reagent is also very efficient. However, the reaction is more difficult to carry out because of induction period and possible frothing. Moreover, there are problems with HPLC monitoring of the efficiency of the destruction limited by precipitation of Fe(III) oxides when using mobile phase with pH higher than 3. (These problems can be overcome by the addition of ethylenediaminetetraacetic acid).

The degradation of Amsacrine and Azathioprine with hydrogen peroxide is not efficient for practical purposes. Complete degradation was achieved for Asparaginase and Thiotepa. For these two compounds this method can be used as second choice if the drug is not mixed with other antineoplastic agents.
The results obtained indicate that Thiotepa and Asparaginase quickly decompose under acidic conditions which offers another possibility for their destruction. This possibility will be further studied.

This investigation is being extended to decontamination of faeces and blood plasma and to further antineoplastic agents used in cancer treatment and the new results will be published as soon as the experimental data are available.

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