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The stability of ifosfamide in aqueous solution and its suitability for continuous 7-day infusion by ambulatory pump

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Summary. Dose fractionation is known to reduce the toxicity of ifosfamide and also results in an increased production of alkylating metabolites. Administration by slow infusion using the convenience of ambulatory pumps is therefore of interest. We used HPLC to investigate the stability of ifosfamide in aqueous solution (either alone, solution A, or mixed with mesna, solution B) under various conditions over a 9-day period. At both ambient temperature in daylight and 27° C in a dark environment, there was no evidence of ifosfamide decay in either solution. However, at 37° C in a dark environment, a fall was detected in both solutions, which at 9 days amounted to a loss of 7% of the amount of ifosfamide present at time zero. At 70° C, levels of ifosfamide in both solutions fell within 72 h to markedly lower levels than controls, thus confirming that the methods used were indicative of stability. We conclude that ifosfamide, either alone or mixed with mesna, is stable for 9 days at temperatures up to 27° C; even at 37° C, the measured loss is small. The continuous infusion of ifosfamide over 7 days by ambulatory pump is now a practical proposition.

Introduction

Ifosfamide is an alkylating oxazaphosphorine used in the treatment of lung cancer, soft tissue sarcoma, osteosarcoma and non-Hodgkin's lymphoma [1]. Its main toxic effect occcurs in the urothelium, causing haemorrhagic cystitis and renal impairment, but this can virtually be abolished by the simultaneous administration of mesna [2], which binds to acrolein, a metabolite of ifosfamide and one of the putative urotoxins. Other toxicity includes myelosupression, nausea and vomiting, and a neurological syndrome characterised by drowsiness, confusion and lethargy [1]. Fractionation of the dose over 3–5 days is common in clinical practice and has been found to be a tolerable and effective method of administration. However, since the manufacturer's instructions state that ifosfamide should be freshly prepared every 8 h, this approach has required daily hospital visits or an in-patient stay. Continuous slow infusion using an ambulatory infusion pump would be an attractive proposition, but an obvious prerequisite is that ifosfamide must demonstrate appropriate stability. The aim of this study was to investigate the stability of ifosfamide using HPLC.

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Materials and methods

Solution A: ifosfamide alone. In accordance with the manufacturer's instructions, 6.25 ml normal saline was added to each of four glass vials containing 500 mg ifosfamide (Mitoxana; Boehringer Ingelheim, Bracknell, UK) and dissolved by agitation.

Solution B: ifosfamide and mesna. For the preparation of this solution, 1.5 ml normal saline and 4.75 ml normal saline containing 0.475 g mesna (Uromitexan; Boehringer Ingelheim, Bracknell, UK) was added to each of four glass vials containing 500 mg ifosfamide and dissolved by agitation.

One vial each of solutions A and B was reserved for the preparation of control samples. A total of 12 aliquots of 300 µl each were removed from each vial, placed in separate microtubes and frozen at –20° C until required. The remaining three vials were air-sealed with a rubber septum and then exposed to the following conditions: (1) ambient temperature (range, 18°–24° C) in daylight, (2) 27° C in a dark environment and (3) 37° C in a dark environment. On days 0–4 and 7–9, 25 µl of each test solution was transferred into a clean tube using a Hamilton syringe, and 175 µl mobile phase was added using a positive displacement pipette. In addition, one control sample of each solution was thawed and a 25-µl aliquot was treated in the same way.

HPLC conditions were as previously described [5], with the exception of the mobile phase, which was modified to a 40:60 (v/v) mixture of acetonitrile and water. Then, 10-µl volumes of both control samples and six test samples were injected onto the HPLC column in random order. The areas under the ifosfamide peaks were measured using a Hewlett Packard 3388A integrator linked to the UV (190 nm) absorbance monitor. The procedure was repeated, also in random order, to obtain a duplicate result for each sample. All vials were weighed after the samples had been withdrawn on day 9 and again 2 weeks later to check for loss of

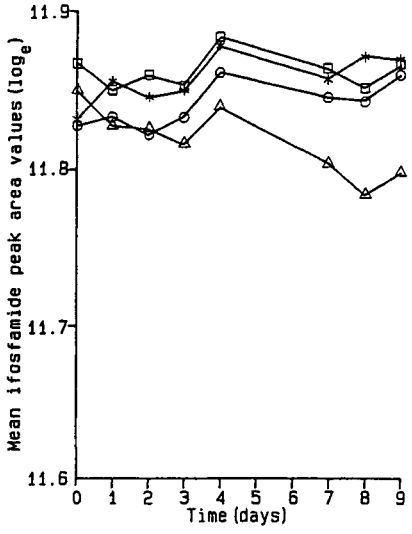

Fig. 1. Mean ifosfamide peak area values ($\log_e$) for solutions A and B subjected to four different conditions for 9 days. *, –20° C; ○, ambient temperature in daylight; □, 27° C in a dark environment; △, 37° C in a dark environment. $P = 0.001$

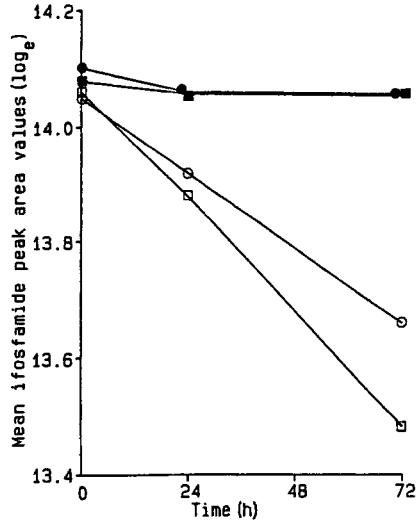

Fig. 2. Mean ifosfamide peak area values ($\log_e$) for solutions A and B exposed to either ambient temperature or 70° C for 72 h. Solution A: ●, ambient temperature; ○, 70° C. Solution B: ■, ambient temperature; □, 70° C. $P < 0.00005$

solvent by evaporation. Finally, all remaining solutions were tested for evidence of bacterial contamination.

To confirm that the methods used were indicative of stability, the experiment was repeated with one vial each of solutions A and B exposed to ambient temperature and 70° C for 72 h. Duplicate samples were removed in random order at time 0, 24 and 72 h and subjected to HPLC as described above.

Statistics. The mean peak area values for ifosfamide were transformed using natural logarithms and then subjected to analysis of variance with repeated measurements.

Results

A time/solution interaction was not detected ($P = 0.5$), indicating that solutions A and B behaved in a similar way over time, and there was also insufficient evidence to suggest a difference between the two solutions irrespective of time ($P = 0.09$). A time/condition interaction was identified ($P = 0.001$), and inspection of the data presented graphically (Fig. 1) suggests that at 37° C in dark environment, there was a decline in the amount of ifosfamide measured by HPLC over the 9-day period, which amounted to 7% of time 0 values on day 9.

Exposure of both solutions to 70° C for 72 h led to a reduction in ifosfamide peak areas to 68% (solution A) and 56% (solution B) of time 0 values. This contrasts with a reduction to 95% (solution A) and 97% (solution B) of time 0 values after 72 h at ambient temperature and represents a highly significant difference ($P < 0.00005$). These data are presented graphically in Fig. 2.

There was no change in the weight of the septum-sealed vials during a 2-week period at the completion of the experiment, and bacteriological screening failed to detect contamination in any of the vials.

Discussion

Ifosfamide is used in the treatment of a variety of tumours [1]. Urothelial damage is the dose-limiting toxicity, but this effect can virtually be abolished by the concurrent administration of mesna [2]. Urothelial damage can also be reduced by dose fractionation, which also results in less haematological toxicity [6]. Moreover, recent studies [3, 4] have shown that dividing the dose over 5 days results in an increased clearance of ifosfamide from the blood and increased levels of alkylating metabolites, effects that probably result from the induction of hepatic microsomal enzymes. Fractionation of the ifosfamide dose is therefore probably important for both limitation of toxicity and enhancement of drug activity, and on this basis, continuous infusion over several days might be expected to be an ideal mode of administration. Mesna is known to be chemically stable for very prolonged periods, with a shelf life of 5 years for the aqueous solution (Boehringer Ingelheim Ltd, unpublished data). There is also no evidence to suggest any interaction between ifosfamide and mesna in vitro [7]. Therefore, subject to the stability of ifosfamide, the infusion of a mixture of these drugs over several days appears feasible.

The results of this study indicate that neither in daylight at room temperature nor at 27° C in a dark environment does a loss of ifosfamide occur from solutions of either ifosfamide alone or ifosfamide plus mesna over a 9-day period. At 37° C in a dark environment a 7% fall in the concentration of ifosfamide in both solutions was detected, which, in the absence of bacterial contamination, we suggest was due to physico-chemical degradation of the drug. We are confident that the methods used were indicative of stability, because a very marked fall in the level of ifosfamide was noted after solutions A and B were heated to

70° C for 72 h. We are also certain that there was no evaporation from the vials, because their weight remained constant over a 2-week period on completion of the study. This is important because a loss of solvent would result in a rising concentration of ifosfamide in the vials, a process that might mask any decrease in the amount present due to drug instability. It is noteworthy that the present studies were first carried out using polyvinylchloride infusion pouches (Pharmacia Deltec Inc., St. Paul, Minn) to contain the drug solutions, but interval weighing on completion of the experiment revealed significant weight loss, presumably due to evaporation through a membrane that was not completely impermeable, and the experiment was therefore repeated using sealed glass vials.

As discussed, the continuous infusion of ifosfamide is attractive from a pharmacokinetic standpoint and might result in less toxicity and an increased anti-tumour effect. We conclude that ifosfamide in aqueous solution, either alone or mixed with mesna, is stable for at least 9 days at body-surface temperature and suggest that the continuous infusion of this drug over 7 days using ambulatory infusion pumps in out-patients is now a practical proposition.

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