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Note

Determination of ifosfamide in plasma by gas chromatography with nitrogen detector

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Ifosfamide (IF, Fig. 1) is a structural analogue of cyclophosphamide (CP) possessing alkylating activity after being enzymatically metabolised to cytotoxic compounds. It is used similarly to CP in the treatment of malignant diseases. It differs from CP in that one of the chloroethyl groups is transferred from the extracyclic to endocyclic nitrogen. The dose of IF varies depending on the clinical indication and the regimen employed. Usually it is given intravenously (injection or infusion) in courses of daily doses over three to ten days. Courses may be repeated after two to four week intervals.

Few analytical procedures have been reported for quantitation of IF following human administration, however, because of its structural similarity with CP, it has often been used as internal standard (I.S.) in the gas chromatographic (GC) determination of CP in biological samples as a trifluoroacetyl (TFA) derivative [1–3]. The derivative is detected either by flame ionization detection (FID), electron-capture detection (ECD) [1], or nitrogen–phosphorus detection (NPD) [2,3]. Holdiness and Morgan [4] have published a GC method for IF based on the formation of a heptafluorobutyric anhydride derivative and ECD. Recently a GC method using NPD for underivatised IF has been reported [5], but using this method we were unable to obtain the necessary reproducibility presumably because of on-column decomposition of the drug. We now describe a GC method for IF in which it is converted to its TFA derivative and detected by NPD. Although the experimental conditions are similar to those described for CP assay [1,2], the method is properly validated with respect to IF and is used in its determination in clinical samples.

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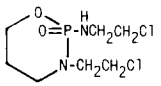

Fig. 1. Structure of ifosfamide.

EXPERIMENTAL

Chemicals and reagents

Analytical-reagent-grade sodium hydroxide and ethyl acetate were purchased from BDH (Poole, U.K.), TFA from Sigma (Poole, U.K.) and IF and CP powders (supplied in sealed vials) from WB Pharmaceuticals (Bracknell, U.K.).

Apparatus

A Model 8310 gas chromatograph (Perkin-Elmer, Beaconsfield, U.K.) equipped with a nitrogen–phosphorus detector and a glass column (2 m × 3 mm I.D.) packed with 3% OV-17 on Gas Chrom Q was used in conjunction with a Model BD 40 recorder (Kipp & Zonen, Delft, The Netherlands). The following GC conditions were applied: column temperature, 230°C; injection port temperature, 300°C; detector temperature, 330°C; nitrogen flow-rate, 30 ml/min; hydrogen and air flow-rates, 16 and 390 ml/min, respectively; nitrogen–phosphorus detector bead setting, 3. The detector was operated in N/P mode.

Procedure

To a stoppered centrifuge tube 0.2 ml plasma sample and 0.2 ml I.S. (50 mg/l CP in water) were added. The mixture was made alkaline with 0.2 ml of 1 *M* sodium hydroxide and extracted with 4 ml ethyl acetate followed by centrifugation at 750 *g* for 5 min. The organic layer was transferred to a 15-ml reaction tube with PTFE-lined screw cap, and was evaporated to dryness at 60°C in a Gyrovap vacuum evaporator (Howe, London, U.K.). To the dried residue were added 100 μl TFA and 100 μl ethyl acetate. The screw cap was tightly closed and derivatisation was completed by heating the mixture to 70°C in a heating block (Techne, Cambridge, U.K.) for 20 min. After cooling, the contents of the tube were evaporated to dryness under vacuum at 60°C. The residue was dissolved in 200 μl ethyl acetate, and 1 μl was injected into chromatograph.

Standard solutions of IF ranging from 0.5 to 50.0 mg/l ($n=6$) were prepared in drug-free plasma. Each standard (0.2 ml) was assayed according to the procedure described. The peak-height ratio of drug to I.S. was plotted against concentration and the calibration graph was used to measure the concentration of IF in the patient samples.

RESULTS AND DISCUSSION

The chromatogram of a plasma sample obtained from a patient is shown in Fig. 2. The retention times for IF and CP are 150 and 240 s respectively. The sharp peaks produced by TFA derivatives fascilitate peak-height measurement. The calibration graph was linear within the above-mentioned concentration range but

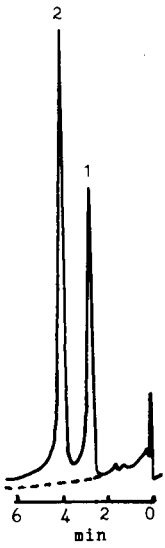

Fig. 2. Chromatogram of plasma sample obtained from a patient containing 20 mg/l IF (1) and 50 mg/l CP (2) as an internal standard. The broken line shows a trace from blank plasma.

at higher concentrations (> 50.0 mg/l) deviation from linearity was observed. The regression equation for the calibration graph is $y = 0.032x + 0.0032$, $r = 0.999$. Good reproducibility of the method was indicated when ten separate determinations of plasma samples containing 10.0 and 40.0 mg/l IF gave within-day coefficients of variation (C.V.) of 3.9 and 1.2% and day-to-day C.V. of 8.2 and 3.7%, respectively. The limit of detection (signal-to-noise ratio of 3:1) of this assay was found to be 0.5 mg/l. Percentage recoveries of IF from plasma were calculated at each calibration point by comparing the peak-height ratios of plasma samples containing known amounts of IF with those of corresponding aqueous solutions. The average recovery of IF in the calibration range was $84 \pm 6\%$. Whenever a batch of samples was analysed, calibration standards were also included in the run to allow a standard curve to be constructed. Furthermore, two quality-control (QC) samples at 10.0 and 30.0 mg/l were run along with each batch of samples. For each batch the results of QC samples were within 10% of known values. No peaks were observed at the retention times of drug and I.S. from the blank plasma nor from dexamethasone, mesna, metoclopramide, morphine and prednisolone which were co-administered with IF. When stored at -20°C, the plasma samples were stable for at least four weeks.

To investigate the correlation between plasma levels of IF and central nervous system (CNS) toxicity, we monitored the steady-state plasma levels of IF (Fig. 3) in patients who received IF as a three-day infusion for the treatment of cervical cancer. Although no such correlation was found in our study, the possibility of CNS toxicity with IF therapy exists particularly in patients with impaired renal excretion. Further work needs to be done in this area. The results, however, demonstrate the usefulness of this method for determining IF in plasma. The use of a nitrogen–phosphorus detector which gives selective response to nitrogen/phos-

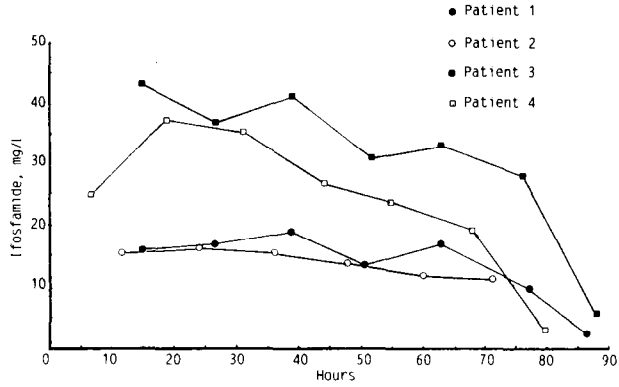

Fig. 3. Plasma profiles of ifosfamide from four patients during the infusion period. Dosing regimen: patients 1 and 2 were given 4.5 g, and 3 and 4 were given 9.0 g IF as infusion over 72 h.

phorus-containing compounds simplifies sample clean-up procedure and gives fewer interfering peaks compared to a GC–ECD method. Although ECD is more sensitive than NPD, the sensitivity of the latter is adequate for IF assay. The proposed method therefore can be used as a useful alternative to other GC methods for investigations concerning IF therapy.

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