Journal of Chromatography, 145 (1978) 315—318
Biomedical Applications

CHROMBIO. 102

Note

Simple and sensitive method for the determination of cyclophosphamide by means of a nitrogen—phosphorus-selective detector

TERENZIO FACCHINETTI, MAURIZIO D'INCALCI, GIAMPIERO MARTELLI, LAVINIA CANTONI, GIORGIO BELVEDERE and MARIO SALMONA

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan (Italy)

(First received March 29th, 1977; revised manuscript received July 4th, 1977)

Cyclophosphamide (Cy) (Fig. 1) is widely used as an anti-tumoral agent in various clinical protocols [1]. The need for information on the bioavailability of this compound makes it important to have at our disposal sensitive analytical methods for measuring Cy plasma levels. Pantarotto et al. [2] have published a Gas Chromatographic (GC) method based on derivatization of the drug with trifluoroactive anhydride. Although this method satisfies the need for high sensitivity, apart from derivatization problems, it also calls for an expensive electron capture detector.

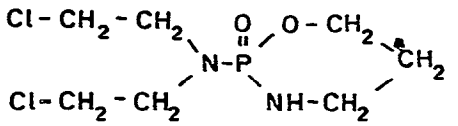

Fig. 1. Structure of cyclophosphamide.

In this paper we present a GC method using a nitrogen—phosphorus-selective detector (NPSD) which can detect as little as 10 ng/ml of the compound without any derivatization. It is possible to measure plasma levels of patients treated with a single intravenous dose of 100 mg of Cy.

MATERIALS AND METHODS

Chemicals

Cyclophosphamide, as the hydrate salt, was kindly donated by the Chester Beatty Institute (London, Great Britain). Imipramine, used as internal stan-

dard, was obtained from Ciba-Geigy (Milan, Italy). All reagents were of the highest purity grade.

GC conditions

GC analysis was carried out with a Fractovap 2300 apparatus (Carlo Erba) equipped with an NPSD (Carlo Erba). A glass column, 2 m × 4 mm I.D. packed with 100—120 mesh Gas-Chrom Q coated with 3% OV-17, was used. The standard operation conditions were: column oven temperature 250°, injector temperature 300°, detector temperature 300°. The gas flow-rates were: nitrogen 40 ml/min; hydrogen, 35 ml/min; air, 220 ml/min.

Extraction procedure from plasma and urine

Plasma and urine were extracted twice with ethyl acetate in the ratio 1:5 (v/v). The solvent phases were collected and evaporated to dryness in a water-bath at 40° under a gentle stream of nitrogen. The samples were submitted to GC analysis adding the necessary volume of ethanol containing 20 μg/ml of imipramine, as internal standard. After the samples were taken up, 1 μl of this solution was injected into the gas chromatograph.

RESULTS

Fig. 2 shows the chromatogram of a plasma blank and of a sample containing

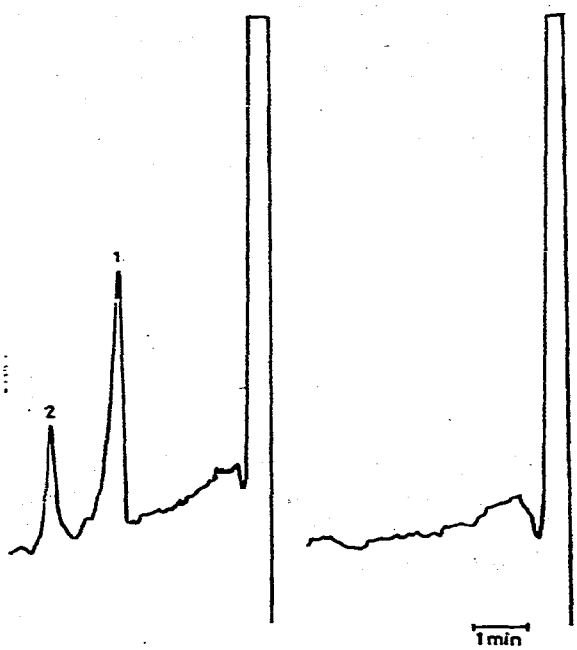

Fig. 2. Gas chromatogram of a blank from plasma (right) and of a biological sample (left) containing 3 μg/ml cyclophosphamide together with 20 μg/ml of the internal standard, imipramine. 1, Cyclophosphamide; 2, imipramine.

about 3 μg/ml of Cy. It is noteworthy that urine extracted with ethyl acetate caused no important peak disturbance in the analysis.

Calibration curves showed a linear response from 0.05 to 0.50 μg/ml. At a concentration of 0.1—10 μg/ml the recovery of Cy from plasma and urine was about 88%. The extractions were carried out on 1 ml of plasma, and on 10 ml of urine.

Fig. 3 shows the disappearance curves of cyclophosphamide from the plasma of three patients after intravenous injection of 100 mg of the drug. Concentration vs. time fit well in a bioexponential function. Analysis of regression shows a high significance ($P < 0.01$).

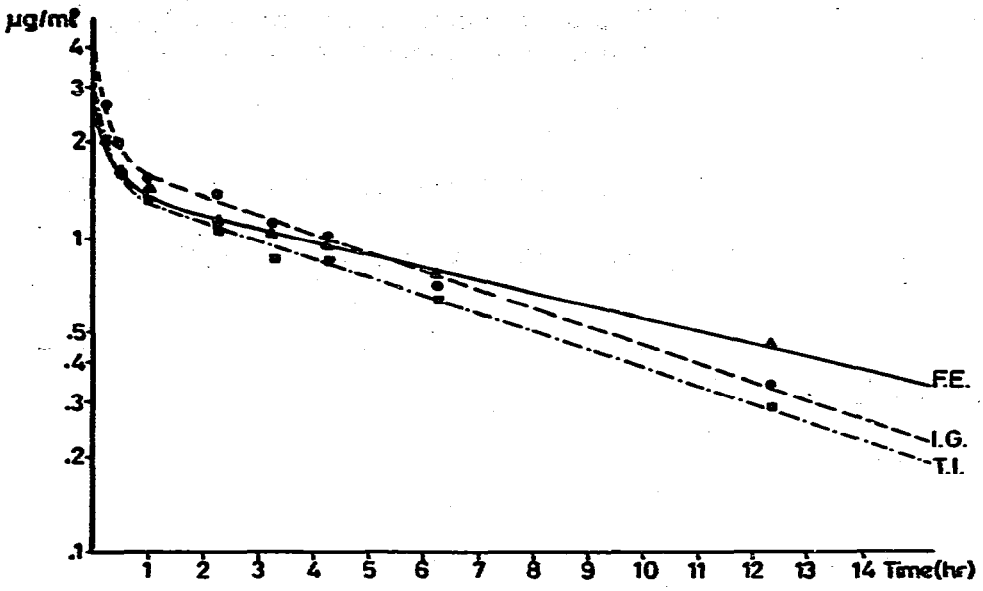

Fig. 3. Plasma levels of cyclophosphamide (μg/ml) in three patients after a single intravenous injection of 100 mg of the drug.

DISCUSSION

To date, the NPSD has mainly been employed to determine organophosphorus pesticides, although it has also been widely used for measuring the levels of some anti-epileptic drugs [3—5]. Its major feature, selectivity for nitrogen and phosphorus, helps overcome one of the biggest problems of quantitative assay: sample cleaness. From a general point of view, extraction with a polar solvent, such as ethyl acetate, from plasma and especially from urine may produce interfering peaks during the GC analysis. With our experimental conditions, however, we were able simultaneously to achieve high recovery of the drug and clean biological samples. Furthermore, the simplicity of the extraction and analytical methods makes it possible to perform about 30—40 determinations per day.

Table I shows the pharmacokinetic parameters in the three patients studied. The mean half-life is 5.87 ± 0.74 h; the volume of distribution (V_d) is 62.2 ± 4.3 l. Total body clearance (ClB = $V_d \times \beta$)* is 124.5 ± 11.1 ml/min. These values are in good agreement with those described by other investigators using ^{14}C-labelled Cy [6,7].

Study of the mechanism of action of Cy depends on our knowing the levels of some of its metabolites, which are the true active compounds [8—10]. However, these must be derivatized in advance to permit their detection. It is also important to note here that most reagents available for derivatization contain one or more nitrogen atoms which saturate the detector since they are generally added in large excess. Studies are in progress in our laboratory in order to stabilize some of the Cy metabolites and evaluate then quantitatively by means of the NPSD, since we believe that this detector may be a useful tool.

TABLE I

PHARMACOKINETIC PARAMETERS OF CYCLOPHOSPHAMIDE IN THREE PATIENTS

Each patient was treated once (intravenously) with 100 mg cyclophosphamide.

Patient	Half-life	V_d (l)	ClB (ml/min)
F.E.	7.36	68	106.5
I.G.	5.08	53.7	122.1
T.I.	5.18	65.1	145.0
Mean ± S.E.	5.87 ± 0.74	62.2 ± 4.3	124.5 ± 11.1

ACKNOWLEDGEMENTS

The authors are grateful for the financial help of Comm. G. Martinelli (Bergamo), which enabled Terenzio Facchinetti to spend six months at the Chester Beatty Institute, London, Great Britain.

REFERENCES

1 R.B. Livingstone and S.K. Carter, Single Agents in Cancer Chemotherapy, Plenum, New York, 1970.
2 C. Pantarotto, A. Martini, G. Belvedere, M.G. Donelli and A. Frigerio, Cancer Treat. Rep., 60 (1976) 493.
3 J.H. Goudie and D. Burnett, Clin. Chim. Acta, 43 (1973) 423.
4 C.V. Abraham and H.D. Joslin, Clin. Chem., 22 (1976) 769.
5 F.L. Vandemark and R.F. Adams, Clin. Chem., 22 (1976) 1062.
6 C.M. Bagley Jr., F.W. Bostick and V.T. DeVita Jr., Cancer Res., 33 (1973) 226.
7 H.T. Mouridsen, O. Faber and L. Skovsted, Cancer, 37 (1976) 665.
8 T.A. Connors, P.J. Cox, P.B. Farmer, A.B. Foster and M. Jarman, Biochem. Pharmacol., 23 (1974) 115.
9 N. Brock, R. Gross, H.-J. Hohorst, H.O. Klein and B. Schneider, Cancer, 27 (1971) 1512.
10 R.F. Struck, Cancer Res., 34 (1974), 2933.

*β= Final slope of the biphasic plot based on the equation for a two-compartment model.