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Pharmacokinetics of Intravenous Cyclophosphamide in Man, Estimated by Gas-Liquid Chromatography

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Summary. *A simple gas chromatographic assay utilising alkali flame ionisation detection is described for the estimation of cyclophosphamide as its trifluoroacetate derivative from plasma. Examination of five patients following intravenous cyclophosphamide gave values of 8.9 h (SD 2.7) for the half-life and 0.061 liters/h/kg (SD 0.011) for whole-body clearance of the drug.*

Introduction

Cyclophosphamide is one of the most widely employed of all cytotoxic agents in clinical practice. It undergoes extensive metabolism following entry into the body into a number of cytotoxic metabolites (Fenselau et al., 1977), and although the parent compound is not cytotoxic in itself, its estimation is a convenient point from which to initiate investigation into the clinical pharmacokinetics of this alkylating agent. Assays published hitherto for its estimation in plasma have relied mainly upon the use of radiolabelled drug (Cohen et al., 1971; Bagley et al., 1973). Thin-layer chromatographic assays yield non-linear calibration curves and reproducibility, of their results is poor (Norpoth et al., 1973), whilst gas-chromatographic assay based on electron-capture detection is subject to interference from endogenous substances and/or cyclophosphamide metabolites, producing a spurious detector response and poor reproducibility (Pantarotto et al., 1974). A combination of gas chromatography and mass spectrometry (Pantarotto et al., 1976; Jardine et al., 1976) has therefore become the method used by most investigators. The method described below offers a rapid and simple technique for cyclophosphamide estimation, which exploits the relatively inexpensive alkali-flame ionisation detector.

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Materials and Methods

To 0.5 ml of plasma containing cyclophosphamide in a tube with a ground-glass stopper is added 5 µl of the internal standard solution, 400 µg/ml ifosfamide in methanol (ifosfamide was a gift from Prof. Dr. N. Brock, Astawerke A.G., Brackwede, W. Germany), and the mixture is made alkaline with 0.2 ml 1 *N* sodium hydroxide. This was extracted with 3 ml ethyl acetate (Analar) on a Rolamix (Luckhams, Burgess Hill, Sussex) for 5 min and centrifuged at 1000 *g* for 5 min, and the ethyl acetate layer was transferred to 15-ml reaction tubes with PTFE-lined screw tops. This extract was evaporated to dryness under a gentle stream of air in a water bath at 60° C. To the dried residue were added 100 µl ethyl acetate and 50 µl trifluoroacetic anhydride (Sigma). The screw tops were tightly closed and derivatisation was completed by heating to 70° C in a heating block and maintaining at this heat for 20 min. After cooling, the contents of the tubes were evaporated to dryness under a gentle stream of air at 60° C. The residue was dissolved in 100 µl ethyl acetate and 2 µl was injected into the gas chromatograph. The gas chromatograph employed was a Pye Unicam series 104 with an alkali flame ionisation detector and fitted with a 2.4 metre glass column of 0.4 mm internal diameter, packed with 3% OV 17 on Chromosorb W (AW–DCMS), 60–80 mesh. The operating conditions were column temperature 230° C, detector temperature 330° C; gas flow rates were carrier gas (nitrogen) 75 ml/min, hydrogen 45 ml/min, and air 550 ml/min.

Results

Specimen chromatograms are shown in Fig. 1. No interfering peaks were noted in blank plasma samples. There is no interference from cyclophosphamide metabolites. Underivatised cyclophosphamide produces one or two poorly defined peaks, presumably because of decomposition on the column, which is unsuitable for quantitative work. The sharp peaks produced by the trifluoroacetate derivative allow reproducible and accurate quantitation by the peak height ratio technique (Janik, 1975; see also Fig. 1). The use of the alkali-flame ionisation detector, which gives selective responses to nitrogen-containing molecules, overcomes the main problem encountered with the assay by electron-capture detection, and sample

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clean-up procedures can be rapid and simple without decreasing sample cleanliness. The calibration curve was linear over the range 0.05 μg/ml to 100 μg/ml and the lower limit of detection was 0.01 μg/ml. Ten replicate analyses from plasma at concentrations of 15 μg/ml and 50 μg/ml gave coefficients of variation for within assay variability of 4.0% and 4.8%. The variation between assays was 7.9% at 5 μg/ml (n = 17), 7.4% at 10 μg/ml (n = 16), and 7.25% at 20 μg/ml (n = 11).

The relative response of the detector to cyclophosphamide and the internal standard ifosfamide was determined by estimation of the peak height ratio (cyclophosphamide/ifosfamide) for a mixture of 10 μg of each added to 1 ml plasma, which was then extracted and derivatised as described. This yielded a mean of 1.45 (SD 0.033 for 13 independent estimations).

Details of the five patients studied following rapid intravenous injection of cyclophosphamide appear in

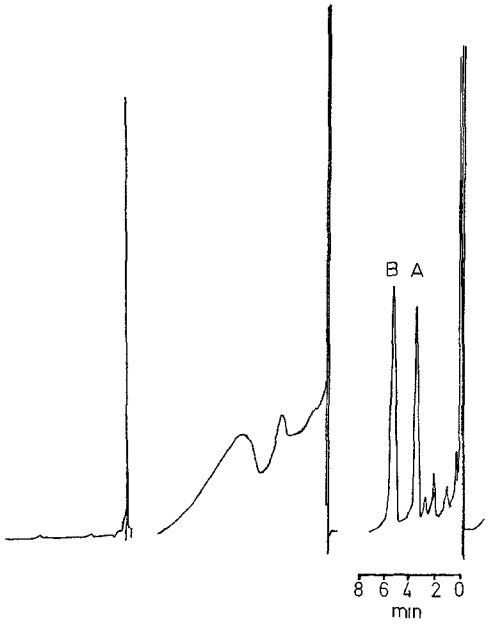

Fig. 1. Gas chromatograms: left, blank plasma; centre, underivatised cyclophosphamide from plasma; right, plasma sample containing 8 μg/ml cyclophosphamide: peak A is ifosfamide, peak B is cyclophosphamide

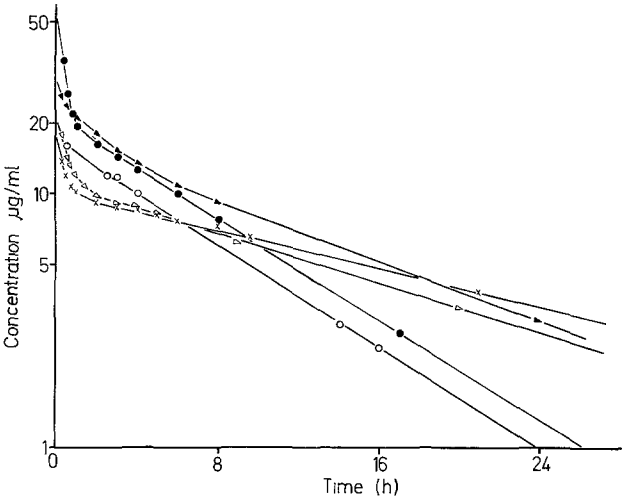

Fig. 2. Plasma cyclophosphamide concentration in 5 patients following intravenous cyclophosphamide. △: patient 1; ×: patient 2; ▲: patient 3; ●: patient 4; ○: patient 5

Table 1. Pharmacokinetic parameters of cyclophosphamide

Patient	1	2	3	4	5
Sex	M	M	M	M	M
Age	56	57	61	66	45
Weight (kg)	83	88	70	48	53
LV dose (mg)	800	800	1000	600	600
Exponential parameters:					
A (μg/ml)	10.43	6.67	10.93	35.81	–
α (h^{-1})	2.37	3.58	3.87	3.72	–
B (μg/ml)	11.38	10.75	13.94	20.40	C_0: 16.11
β (h^{-1})	0.056	0.069	0.070	0.116	k_e: 0.108
$t_{1/2}\beta$ (h)	12.38	10.00	9.94	5.97	6.42
V_c (l/kg)	0.44	0.52	0.57	0.22	–
$Vd\beta$ (l/kg)	0.82	0.84	0.87	0.58	0.70
Clearance (l/h/kg)	0.046	0.058	0.060	0.067	0.076
Concurrent drug treatment	V, P, N	V, P, N	V, P, Ad	V, M, Ac	V, M

Key to drugs: V = vincristine; P = prednisolone; Ad = adriamycin; N = nitrazepam; Ac = alclofenac; M = metoclopramide

Patient 5 data fitted to one-compartment open model: C_0: estimated plasma cyclophosphamide concentration immediately after i.v. injection; k_e: first-order elimination rate constant

Table 1. These patients were being treated for lymphoma. Figure 2 shows the plasma cyclophosphamide concentrations related to time.

Complete 24-h urine collections were available for patients 1, 2, and 5. The elimination of unchanged cyclophosphamide in these patients was respectively 25.1, 25.1, and 20.0 mg, representing approximately 3% of the dose administered. Bagley et al. (1973) state that intact cyclophosphamide excretion in the urine after the first 24 h is negligible. Our figures for excretion fall at the lower end of the range of 2.5%–20% of dose for free cyclophosphamide excretion in urine determined by these workers.

Discussion

The assay of cyclophosphamide with the aid of gas-liquid chromatography with nitrogen detection offers a rapid and specific technique suitable for routine clinical monitoring of plasma cyclophosphamide levels. We have also used the identical method for assay in samples of urine obtained from patients.

As found by other workers (Cohen et al., 1971; Bagley et al., 1973) the plasma disappearance curves (Fig. 2) can be fitted by an open two-compartment pharmacokinetic model described by $Cp = A \exp(-\alpha t) + B \exp(-\beta t)$ with concentration (A, B) and rate (α, β) parameters. In one of our patients (no. 5) a single-compartment model was satisfactory [$Cp = Co \exp(-k_e t)$]. The data was fitted by digital computer with a simplex non-linear optimisation technique (Nelder and Mead, 1965) to generate the lines in Figure 2. The areas under the plasma concentration – time curves (AUC) were calculated by numerical integration and the following pharmacokinetic parameters derived (Gibaldi and Perrier, 1976): The volume of the central compartment V_c = dose/A + B; the apparent volume of distribution during the β phase $Vd\beta$ = dose/(AUC)β; and whole-body clearance = $Vd\beta.\beta$. These calculated values appear in Table 1. These and the range of half-lives during the β-phase ($t^{1}/_{2}\beta = 0.693/\beta$) are similar to those estimated by radioactive tracer techniques (Cohen et al., 1971; Bagley et al., 1973). These results therefore validate the use of this technique for the estimation of plasma cyclophosphamide levels.

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References

Bagley, C. M., Bostick, F. W., Vita, V. T. de: Clinical pharmacology of cyclophosphamide. Cancer Res. **33,** 226 (1973)

Cohen, J. L., Jao, J. Y., Jusko, W. S.: Pharmacokinetics of cyclophosphamide in man. Br. J. Pharmacol. **43,** 677 (1971)

Fenselau, C., Kan, M.-N. N., Rao, S., Myles, A., Friedman, O. M., Colvin, M.: Identification of aldophosphamide as a metabolite of cyclophosphamide in vitro and in vivo in humans. Cancer Res. **37,** 2538 (1977)

Gibaldi, M., Perrier, D.: Pharmacokinetics. New York: Dekker 1976

Janik, A.: What is the best measure of the detector signal? The peak area or the peak height? J. Chromatogr. Sci. **13,** 93 (1975)

Jardine, I., Brundrett, R., Colvin, M., Fenselau, C.: Approaches to the pharmacokinetics of cyclophosphamide (NSC-26271): Quantitation of metabolites. Cancer Treat. Rep. **60,** 403 (1976)

Nelder, J. A., Mead, R.: A simplex method for function minimisation. Computer J. **7,** 308 (1965)

Norpoth, K., Addicks, H. W., Witting, U.: Quantitative Bestimmungen alkylierender Zytostatika auf der DC-Platte mit 4-Pyridincarboxaldehyd-2-benzothiazolylhydrazon (PBH). Arzneim.-Forsch. **23,** 1529 (1973)

Pantarotto, C., Bossi, A., Belvedere, G., Martini, A., Donelli, M. G., Frigerio, A.: Quantitative GLC determination of cyclophosphamide and isophosphamide in biological specimens by means of gas chromatography. J. Pharm. Sci. **63,** 1554 (1974)

Pantarotto, C., Martini, A., Belvedere, G., Donelli, M. G., Frigerio, A.: Studies on the disposition of cyclophosphamide (NSC-26271) in tumor-bearing mice by means of gas chromatography – chemical ionisation – mass fragmentography. Cancer Treat. Rep. **60,** 493 (1976)

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