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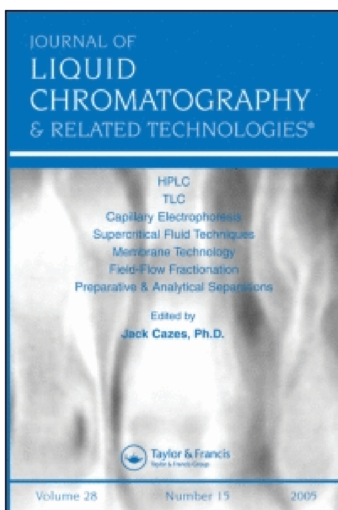

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Original Article

LIQUID CHROMATOGRAPHIC ANALYSIS OF PLASMA MELPHALAN WITH AMPEROMETRIC DETECTION IN A PATIENT WITH BREAST CANCER

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ABSTRACT

We developed a liquid chromatographic method for the measurement of melphalan in plasma and studied a patient with breast cancer, given melphalan before an Autologous Bone Marrow Transplant (ABMT). A reverse phase C 18 column with isocratic elution and amperometric detection was used. The potential was set at + 0.95V. Sample pretreatment involved extraction of the drug with ethyl acetate-ethanol. The method is sensitive and precise and was used to study the pharmacokinetics of melphalan.

INTRODUCTION

Melphalan (p-(di-2-chloroethyl) amino-L-phenylalanine) is an alkylating agent used in the treatment of multiple myeloma, ovarian carcinoma and as

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adjuvant therapy for breast cancer (1-4). In clinical practice oral as well as intravenous administration of this drug is routine. Particularly when combined with other therapy, melphalan may have a beneficial effect in the treatment of some neoplastic diseases. Very promising results were obtained by using melphalan in Regional Perfusion and Autologous Bone Marrow Transplantation (ABMT) (5-6).

Earlier experience with melphalan pharmacokinetics in local perfusion showed rapid decomposition (7), necessitating constant adjustment of the dose to avoid ineffective or toxic levels. Melphalan pharmacokinetics were studied in the plasma of a patient with breast cancer who was treated with Autologous Bone Marrow Transplantation and combined chemotherapy.

This paper concerns the determination of the time-concentration profile of melphalan in plasma using HPLC and an amperometric detector. Procedures for the quantitation of melphalan have been recently reviewed (8). Concentration in biological material have been measured mainly by reversed-phase HPLC and a number of methods are available that differ in clean-up procedures, solvent composition and the detection mode (9-13). Based on these reports, we report a modified assay for the determination of melphalan in plasma. Our method is the first employing HPLC and electrochemical detection.

EXPERIMENTAL

Reagents

Melphalan and methyl paraben (internal standard I.S.) were supplied by Sigma (St. Louis, Mo., USA) Sodium phosphate monobasic, phosphoric acid (85%), NA_2EDTA and HPLC grade acetonitrile were supplied by Fisher Scientific (Montreal, Canada).

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Chromatographic Conditions

The HPLC system consisted of a Waters M-45 pump (Milford, MA, USA), Rheodyne 7125 injection valve with a 100 ul loop (Cotati, CA., USA), amperometric detector LC-4B with thin layer electrolytic cell and glassy carbon as working electrode and Ag/AgCl (3MKCl) as reference electrode from Bioanalytical System, Inc., (West Lafayette, In., USA). The column was 25 x 0.46 cm I.D. packed with Hypersil C18 5 um particle size, obtained from CSC (Montreal, Canada). The detector was set at +0.95V (vs. Ag/AgCl).

Mobile Phase:

The mobile phase contained 70% 0.1 sodium phosphate monobasic, 1mM Na_2EDTA and 30% acetonitrile (v/v) adjusted to pH 4.0 with 85% phosphoric acid and passed through a 0.45 um nylon filter. The flow rate was set at 1.5 ml/min. The injection volume of the sample was 70 ul.

Standard Control and Sample Preparation.

The Standard and control samples (CS) were prepared by adding appropriate volumes of water-acetonitrile (1:1,v/v) spiked with melphalan to human plasma. Calibration curves ranged from 10 to 1200 ng/ml. The volume added was always smaller than or equal to 5% of total volume of the sample. After aliquoting, 1 ml samples were stored at- $15^{o}C$ until required.

Clinical Pharmacokinetic Study

One patient with breast cancer before Autologous Bone Marrow Transplantation received combination chemotherapy including: melphalan, cyclophosphamide, cisplatin and mitoxantrone, with Melphalan being administered on day-5, and-4 pre marrow reinfusion. She received a high dose of melphalan (70 mg/m^2) intravenously.

Plasma Samples

Blood samples (5-7 ml) were collected in heparinized tubes and immediately placed in the refrigerator. After

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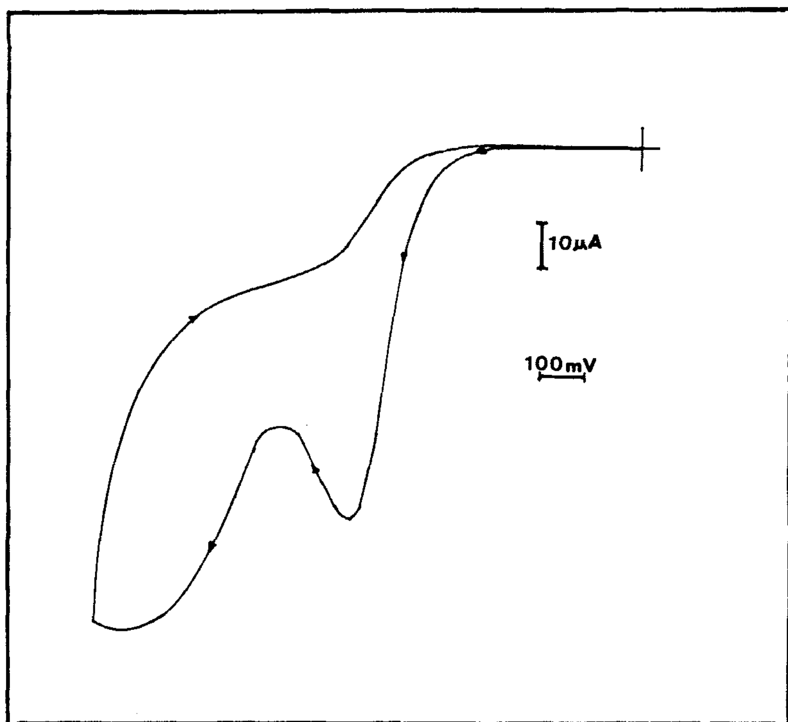

Figure 1 Cyclic voltammogram of 5mM melphalan in the mobile phase. The scan rate was 10mV/s and the initial potential + 0.3V.

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centrifugation(+4°) the plasma fraction was removed and stored at- 15°C until analysis. Blood samples were taken on day-5 before ABMT, at 0.5 hr. before and 0.5,1,2,3,4,5,6,12,18,24 hr. after the end of the melphalan infusion. The patient was again infused with melphalan on day-4 before ABMT and the same procedure as outlined above was followed.

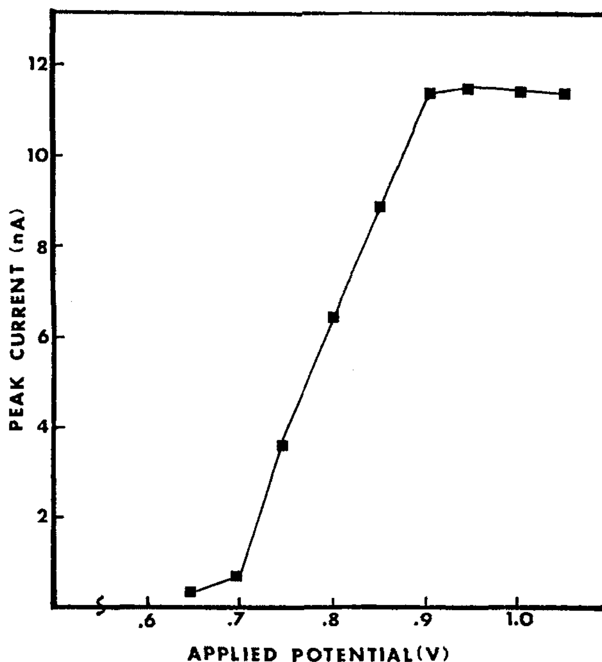

Figure 2. Current-voltage curve for repetitive injection of 50 ng of melphalan. Potentials are vs Ag/AgCl.

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Method of Extraction

Internal standard was added (100 ul of methyl paraben, 50 ug/ml) to 1 ml of standard, control sample or clinical samples. After adding 7 ml of ethyl acetate-ethanol (95/5 v/v), the tubes were shaken for 20 minutes on a reciprocal shaker at 150 rpm and then centrifuged in the cold (+4°) for 5 minutes at 1000g. The upper organic layer was transferred into culture tubes and evaporated to dryness under a stream of nitrogen at room temperature. The residue was dissolved in 100 ul of mobile phase and 70 ul of the solution was injected into the system.

TABLE I

Representative Precision and Accuracy of The Plasma Melphalan Assay

Nominal concentration (ng/ml)	Concentration found (mean + S.D.) (ng/ml)	Coefficient of variation (%)	Percentage of nominal concentraton
10.0	10.15 ± 0.47	4.65	101.54
100.0	97.0 ± 2.31	2.38	97.0
400.0	403.4 ± 15.85	3.93	100.85
1200.0	1234.2 ± 51.53	4.18	102.85

Calibration parameters (5 curves) were calculated by linear regression analysis; correlation coefficient > 0.992.

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RESULTS AND DISCUSSION

Electrochemical Behaviour of Melphalan

The first voltammetric data on melphalan oxidation were reported by Barek and co-workers(14). The oxidative behaviour of melphalan at the glassy carbon electrode was examined by cyclic voltammetry and hydrodynamic voltammetry. A sample response curve (a voltammogram) is illustrated in Figure 1. The mechanism of anodic oxidation of melphalan is complex, although the first step is usually the removal of an electron to give a radical-cation. In the analysis of melphalan the electron is invariably removed from a hetero-atom, commonly nitrogen, the potentials used being insufficient to oxidize the carbon skeleton of the molecule

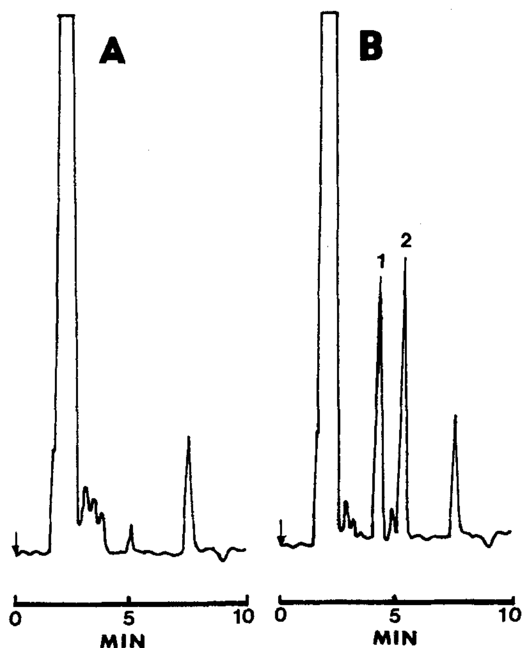

Figure 3. Representative chromatograms of melphalan in plasma. A-drug-free plasma, B-plasma obtained 2 hours after 70 mg/m^2 melphalan (1) administration. Chromatographic conditions: Potential + 0.95V, Mobile phase-70% 0.1M sodium phosphate monobasic, 1mM Na_2EDTA and 30% acetonitrile (v/v),pH 4.0, flow rate 1.5 ml/min.

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Hydrodynamic voltammetry was used to determine the optimum detector potentials for detection of melphalan. The hydrodynamic voltammogram for the oxidation of melphalan is shown in Figure 2. The limiting current plateau is a potential greater than 0.93 V. A potential of +0.95V was used to detect .

TABLE II

Pharmacokinetic Data of Melphalan After Intravenous Administration of 70 mg/m^2 Dose.

Parameter	5 days pre ABMT	4 days pre ABMT
Elimination constant (Kel)	0.974	0.947
Elimination half-life ($t_{0.5}$)(min)	42.7	43.9
Area under the curve (AUC 0-5) (ng/hr. ml)	1047.22	905.48
Maximum plasma concentration (Cmax)(ng/ml)	1068.68	816.64

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Precision And Accuracy

A set of eight calibration standards, a control sample and drug free plasma samples were analyzed with every batch of clinical samples. The within-run and between-run precision and accuracy of the assay is shown in Table 1. Linear responses of melphalan and internal standard peak height ratios were observed over concentration range of 10 to 1200 ng/ml. The correlation coefficients between response and drug concentration were equal to or better than 0.992 in five curves. The coefficient of variation was less that 4.65% and deviation from nominal concentration, a measure of accuracy, was less than 1.54% at the lower end of the standard curve. The detection limit (signal to noise ratio 3:1) of the method for melphalan was 5 ng/ml.

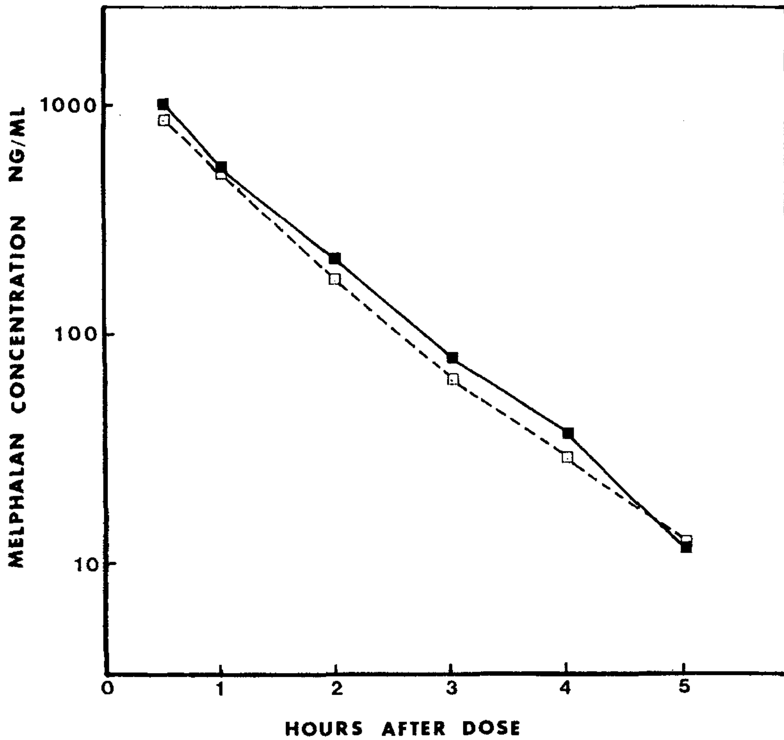

Figure 4. Concentration /time melphalan plasma profiles after intravenous administration of 70 mg/m^2 dose. Full square and solid lines indicate results obtained 5 days pre ABMT. Open square and dotted lines indicate results obtained 4 days pre ABMT.

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Recovery

The recovery of melphalan from plasma at 20 ng/ml was 87.8% (CV=3.6%,n=5)

Chromatography

Figure 3 shows the chromatograms of a drug free plasma sample (A) and a patient sample (B) taken 2 hours

after the administration of melphalan (70 mg/m^2 intravenously). The retention time for melphalan was 4 min. and for methyl paraben (I.S.) 5 min. The analysis time did not exceed 10 minutes.

Pharmacokinetic Data

The following parameters were determined: Area under the curve (AUC), peak concentration (Cmax), elimination rate constant (Kel) and elimination half-life ($t_{0.5}$) (15).

The trapezoidal method was used to calculate the area under the curve until the final detectable plasma concent- tration. The residual area extrapolated to infinity, was added to the area under the curve, calculated by the division of the final concentration by Kel. The apparent elimination half-life was calculated directly from Kel. The results are summarized in Table 2, while the plasma profiles are shown in Figure 4. The pharmocokinetic data for the melphalan obtained by this method correspond to those reported by Friberg et al (16), with a half life of 42.7 minutes agreeing well with the value of to 52.5 minutes in the quoted study. The concentration of melphalan 6 hr. after infusion was < 5 ng/ml.

In conclusion our method is simple, reproducible and precise. It is specific for melphalan in this patient' plasma, specific for melphalan, since the simultaneous presence of cyclophosphamide, cisplatin and mitoxantrone did not interfere with the detection and measurement of melphalan.

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REFERENCES

(1) R. Alexander, D. Bergsagel and P. Migilore, Blood, 31, 1 (1968).

(2) O. McIntyre, L. Leone and T. Pajak, Blood, 52, 274 (1978).

(3) J. Smith and F. Rutledge, Natl. Cancer Inst. Monogr., 42, 141 (1974).

(4) B. Fisher, P. Carbone, S. Economon, R. Frelick, A. Glass, H. Lerner, C. Redmond, M. Zelen, P. Band, D. Katrych, N. Wolmark and E. Fisher, N. Engl. J. Med., 292, 117 (1975).

(5) E.T. Krementz, M. Campbell in Malignant Melanoma ed. by J.J. Costanzi, Vol.1, pp.225-257 (1983).

(6) M.D. Vincent, T.J. Powles, R. Ch. Coombes and T.J. McElwain, Cancer Chemother. Pharmacol., 21, 255, (1988).

(7) F. Malecki, J.C. Crawhall and R. Staroscik, J. Pharm. Biomed. Anal., 5, 717 (1987).

(8) S. Eksborg, M. Ehrsson, J. Chromatogr., 340, 31, (1985).

(9) S.Y. Chang, D.S. Alberto, L.R. Melnick, P.D. Walson and S.E. Salmon, J. Pharm. Sci., 67, 679 (1978).

(10) A.E. Ahmed and Hsu Ting-Feng, J. Chromatogr. 222, 453, (1981).

(11) C.M. Egan, C.R. Jones and M. McCluskey, J.Chromatogr., 224, 338, (1981).

(12) A.G. Bosanquet and E.D. Gilby, J. Chromatogr. 232, 345 (1982).

(13) H. Ehrsson, S. Eksborg and A. Lindfors, J. Chromatogr., 380, 222, (1986).

(14) J. Barek, A. Bereka and J. Zima, Anal. Lett., 18, 2581, (1985).

(15) M. Rowland and T.N. Tozer in Clinical Pharmacokinetic, Ed. by Lea and Febiger, Philadephia, London (1989).

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(16) S. Friberg, H. Ehrsson, S Eksborg and Ch. Carenfeldt, Cancer Chemother. Pharmacol., 20, 34, (1987).

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