Journal of Chromatography, 339 (1985) 445—450
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2511

Note

High-performance liquid chromatographic determination of 2-[3′-(methoxycarbonylamino)-phenyl]-3-phenyl-6-methoxycarbonylamino-4-(3H)-quinazolone (NSC-251635) in human serum

C. BRASSINNE*, C. LADURON, J.P. SCULIER and A. COUNE

Service de Médecine et Laboratoire d'Investigation Clinique H. Tagnon, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, rue Héger-Bordet 1, 1000 Brussels (Belgium)

(First received May 22nd, 1984; revised manuscript received December 13th, 1984)

2-[3′-(Methoxycarbonylamino)-phenyl]-3-phenyl-6-methoxycarbonylamino-4-(3H)-quinazolone (NSC-251635, I) is a water-insoluble antimitotic compound (Fig. 1).

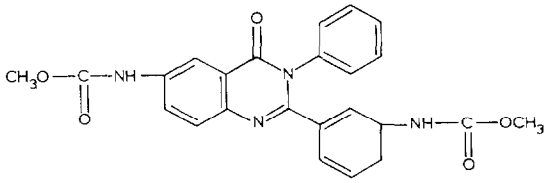

Fig. 1. Chemical structure of I.

Recently, we investigated the use of liposomes (phospholipid vesicles) as carriers for water-insoluble drugs [1, 2] and demonstrated that I was active on L1210 murine leukaemia only after entrapment in liposomes [3]. These results prompted us to determine whether large amounts of liposomes containing I could be infused into man; preliminary results demonstrated that liposome volumes as large as 400 ml could be infused into man by the intravenous route without major side-effects [4]. In order to investigate the main pharmacological parameters of the liposome-entrapped drug, a selective, sensitive and quantitative method of I determination was needed.

This report describes the application of reversed-phase high-performance liquid chromatography (HPLC) to the determination of the concentration of I in human serum samples previously submitted to a quantitative extraction procedure.

0378-4347/85/$03.30

EXPERIMENTAL

High-performance liquid chromatography

A Waters chromatograph was equipped with a Model 6000A solvent delivery system, a Model 710B sample processor and a Model 480 Lambda-Max spectrophotometer operating at 297 nm; peak areas were integrated by a Waters Data Module M 730. A radial compression separation system (module Z) equipped with a Radial-Pak C_{18} cartridge (mean particle size 10 μm) was also supplied by Waters Assoc. (Milford, MA, U.S.A.). The column was preceded by an on-line stainless-steel precolumn (5 cm × 3.9 mm I.D.) packed with Vydac^{TM} 201 RP (particle size 30—44 μm; Macherey, Nagel & Co., Düren, F.R.G.).

Reagents and standards

I was provided by Dr. R. Bierling (Bayer, Wuppertal, F.R.G.), HPLC-grade methanol was purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

A stock solution of I (0.5 mg/ml) was prepared in methanol and working standard solutions (0.05—250.0 μg/ml) were prepared by dilution of the stock solution with methanol.

TABLE I

CHROMATOGRAPHIC CONDITIONS

Parameter	Conditions
Column	μBondapak C_{18} (10 μm)
Mobile phase	Methanol—water (625:375)
Wavelength	297 nm
Flow-rate	3.5 ml/min
Temperature	Ambient
Chromatography time	5 min
Chart speed	0.5 cm/min
Sample size	50 μl

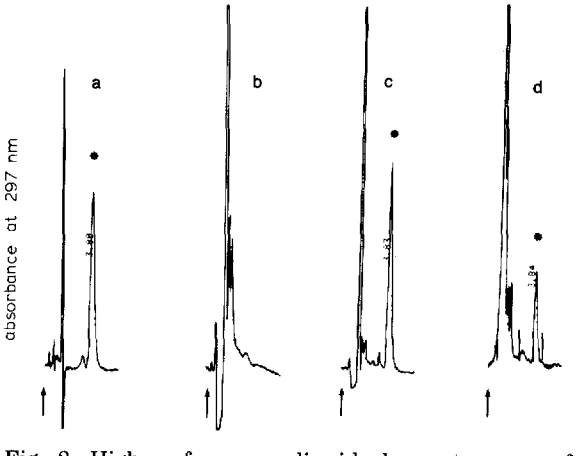

Fig. 2. High-performance liquid chromatograms of I (*): (a) standard (0.005 a.u.f.s.); (b) blank human serum (0.005 a.u.f.s.); (c) control sample (0.02 a.u.f.s.); (d) patient serum sample (0.005 a.u.f.s.). Arrow indicates start of injection.

Control samples were prepared by adding I to pooled drug-free sera to obtain concentrations of I ranging from 0.05 to 250.0 μg/ml.

Blood samples obtained from patients infused with a liposome preparation containing I were centrifuged at 1500 *g* for 10 min and the serum supernatants stored at −20°C until analysis.

Extraction procedure and conditions of analysis

The sample to be tested (1 ml) was mixed vigorously on a vortex mixer with 4 ml of methanol, left at 6–8°C for 30 min, and centrifuged at 1500 *g* for 5 min at 4°C. The clear supernatant was decanted and injected into the liquid chromatograph.

Table I lists the chromatographic conditions used in the analysis. Typical chromatograms for a standard, a serum blank, a control sample and a patient serum sample are shown in Fig. 2.

RESULTS AND DISCUSSION

Analytical variables

Among the different extraction procedures tested, the best drug recovery is obtained using the methanolic extraction.

Fig. 3 shows the absorbance scan of I in the ultraviolet–visible spectrum. The absorption maximum is at 297 nm. At shorter wavelengths more methanol-extractable material is detectable in extracts of biological specimens.

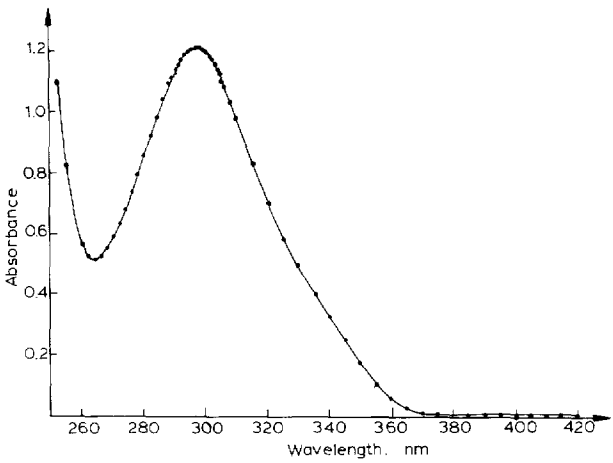

Fig. 3. Absorbance scan of I (25 μg/ml) in methanol.

As shown in Fig. 2, in the chromatographic system the elution profiles of both standard (a) and extracted serum samples (c and d) are similar. In serum, the drug peak is well separated from other detectable compounds and the extract of serum blank (b) shows no interference peak at the retention time of I.

All samples are extracted and chromatographed in duplicate, and the drug concentration is calculated from the peak area of I.

Linearity

There is a linear relation between the concentrations of I in the standard (r = 0.9999) and control serum samples (r = 0.9998) and the recorded peak areas. This linear relation extends to concentrations of at least 500 μg/ml.

Recovery

Known amounts of I were added to drug-free serum to provide concentrations ranging from 0.05 to 250 μg/ml. After duplicate extraction and chromatography of ten samples of each concentration, the peak areas obtained for I were compared to the peak areas obtained by direct injection of corresponding working standard concentrations. Recovery was calculated as

$$\frac{\text{Amount of drug measured}}{\text{Amount of drug added}} (\mu g/ml) \times 100$$

The results summarized in Table II show that a 98.0—100.4% recovery of I is obtained within the concentration range 0.05—250.0 μg/ml of serum.

Repeated assays of serum containing I stored at —20°C for one to twelve months, yielded concentration values identical to those obtained for the fresh serum sample; thus there is no indication of loss of I during this storage period.

TABLE II

RECOVERY STUDY

Concentration of I added (μg/ml)	Concentration of I measured* (μg/ml)	Recovery (%)
0.05	0.049	98.0
0.5	0.502	100.4
1.0	0.992	99.2
2.0	2.002	100.1
5.0	4.931	98.6
10.0	10.027	100.3
20.0	19.965	99.8
100.0	99.703	99.7
250.0	249.750	99.9

*Values represent the mean of ten separate determinations.

TABLE III

BETWEEN-DAY PRECISION

Concentration of I (μg/ml)	Coefficient of variation (n = 12) (%)
0.5	3.4
1.0	2.9
2.0	2.5
5.0	2.0
10.0	2.8
20.0	2.4

Sensitivity

The sensitivity of the assay (i.e. peak height corresponding to twice the baseline noise) was found to be 25 ng/ml.

Precision

The between-day precision was calculated for six concentrations of I in serum. Six samples of each concentration were extracted and chromatographed each day for twelve working days. Table III shows that the method has a satisfactory precision with a coefficient of variation equal to or less than 3.4% for I serum concentrations in the range 0.5—20 μg/ml.

Clinical application

The method described in this paper provides a rapid, selective and sensitive assay for measurement of concentrations of I in human serum. The sensitivity of this method makes it very useful for pharmacokinetic studies of I entrapped in liposomes and administered intravenously to man. The results of such a pharmacokinetic study is shown in Fig. 4. A 225-ml volume of liposomes containing 158 mg of I was infused into a patient over a 2-h period. The peak value of the concentration of I reached after 2 h is followed by a marked decrease until the sixth hour after the start of the infusion; during the following 14 h a very slow decrease in the serum concentration of I occurs, but some drug is still present in the blood 48 h after the start of the infusion.

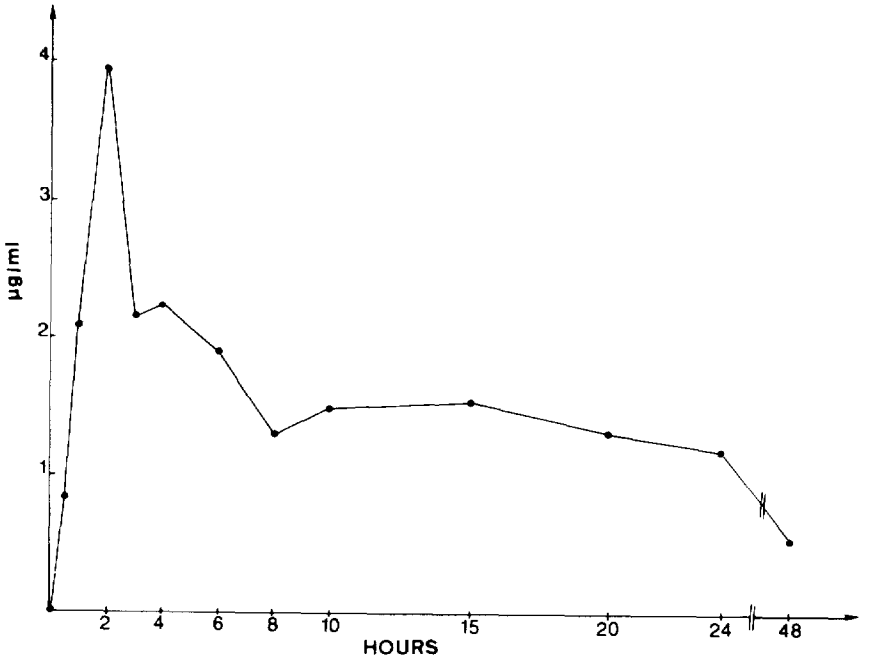

Fig. 4. Blood concentration of I as a function of time after intravenous infusion to man of liposomes containing I.

ACKNOWLEDGEMENT

This work was supported by a grant from the Fonds Cancérologique de la Caisse Générale d'Epargne et de Retraite de Belgique.

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