Journal of Chromatography, 342 (1985) 431—435
Biomedical Applications
Elsevier Science Publishers B V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2628

Note

Determination of N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide in plasma by high-performance liquid chromatography

JEFFREY L. JURLINA* and JAMES W. PAXTON*

Department of Pharmacology and Clinical Pharmacology, University of Auckland School of Medicine, Auckland (New Zealand)

(First received October 31st, 1984; revised manuscript received March 14th, 1985)

We have previously reported a high-performance liquid chromatographic (HPLC) method for the determination of the novel anticancer drug amsacrine, 4'-(9-acridinylamino)methanesulfon-*m*-anisidide in plasma [1], which is suitable for pharmacokinetic studies in rabbits and patients [2, 3]. Although amsacrine is an effective clinical drug for the treatment of various haematological malignancies it has shown disappointing activity against most solid tumours [4]. A new analogue, N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide [I, CI-921, NSC 343 499) has been identified, which has significantly superior activity in various in vitro and in vivo solid tumour test systems, and which may offer a broader clinical antitumour spectrum [5]. This compound will enter phase 1 clinical trials early in 1985. We have developed an HPLC method for the determination of I in plasma which will allow the study of its pharmacokinetics during phase 1 trials, and also in animals.

MATERIALS AND METHODS

The apparatus and materials have been previously described [1]. The internal standard (I.S.) has the same structure as I, but with the methyl in the sulfonanilide group substituted by an ethyl group (Fig. 1). This substitution did

*Present address: Cancer Research Laboratories, University of Auckland School of Medicine, Auckland, New Zealand.

0378-4347/85/$03.30

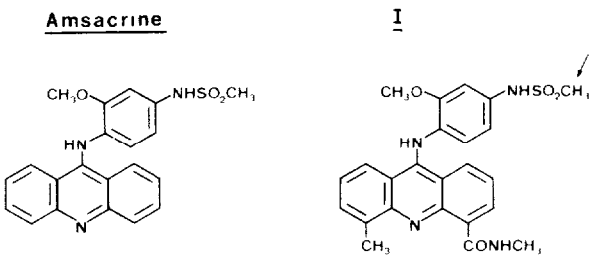

Fig. 1. Structures of amsacrine and analogue (I). The internal standard had an ethyl group substituted for the methyl group indicated on I.

not alter the pK_a of the molecule or its extraction properties. However, the greater lipophilicity will result in a longer retention time on the Radial-Pak C_{18} column. Pure I (the isethionate salt) and the I.S. (the hydrochloride salt) were supplied by Dr. B. Baguley (Cancer Research Laboratory, Auckland School of Medicine, Auckland, New Zealand). Stock solutions (2 mmol/l) were made up in methanol. This stock solution of I was further diluted 1:100 with blood-bank plasma to give a concentration of 20 μmol/l. Further serial dilutions with plasma were made to give the following concentrations: 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2 and 0.1 μmol/l plus a blank plasma. These concentrations were used to construct a calibration curve. The I.S. solution was further diluted 1:100 in methanol to 20 μmol/l and 100 μl were added to each 0.5-ml plasma sample. In addition, pure I was weighed out and added to four large plasma pools to give concentrations of 10, 5, 2 and 1 μmol/l, which were used to determine the accuracy and precision of the method. They were also included in each subsequent assay as quality controls, and to determine the inter-assay precision.

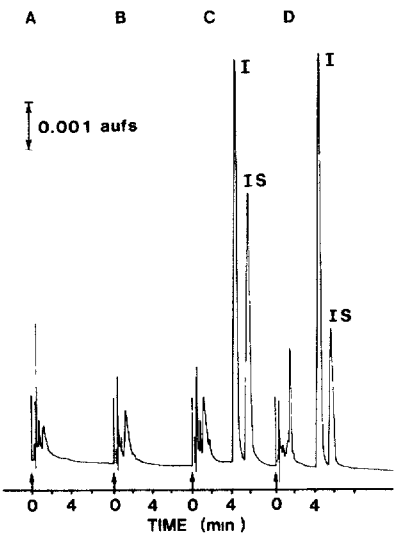

Fig. 2. HPLC traces of extracts of blank human plasma (A), blank rabbit plasma (B), human plasma quality control (5 μmol/l) (C), and post-infusion rabbit sample (D). IS = internal standard.

The clean-up and extraction procedure were the same as for amsacrine [1]. The HPLC mobile phase was prepared by adding 10 ml of stock triethylamine phosphate (TEAP) solution to 990 ml of acetonitrile—water (43:57) to give a final concentration of 0.01 mmol/l TEAP. The chromatographic separations were performed on a Waters 10 × 0.8 cm Radial-Pak C_{18} column (10 μm particle size) with a mobile phase flow-rate of 6.5 ml/min and detection by a Model 440 UV detector at 254 nm. Under these conditions the elution time of I and I.S. were 4.5 and 5.7 min, respectively, with baseline separation (Fig. 2).

RESULTS

A calibration curve for I in plasma ranging from 0.1 to 20 μmol/l was prepared by plotting peak area ratios to concentrations of I. An excellent linear relationship was obtained, represented by the equation $y = 0.242x - 0.055$ ($r = 0.9999$, $p < 0.001$). Using a 0.5-ml plasma aliquot, 0.1 μmol/l was the lowest concentration that could be measured with acceptable precision, that is a coefficient of variation of less than 7% for six repeated measurements within one assay. The accuracy of the measurement of this lowest concentration was also acceptable, lying between 85 and 115% of the true value, i.e. a 85–115% recovery. Estimates of the intra-assay precision and the accuracy of the method over the range 1—10 μmol/l are given in Table I. The precision of the method was good, with the coefficients of variation for six determinations of each concentration all being less than 2.7%. The accuracy of the method over this range was also acceptable, with the mean concentrations determined lying within 99—114% of their true values. The reproducibility of the method was tested over a three-month period by including aliquots of the four plasma pools in fifteen consecutive assays. The results are presented in Table II and indicate good reproducibility with the coefficients of variation for the fifteen determinations all being less than 5.3%. From this data, it also was apparent that I is stable in plasma stored frozen at —20°C for at least three months.

No peaks interfering with I or I.S. were observed on extraction of human or rabbit plasma (Fig. 2). A number of other anticancer drugs including adriamycin, chlorambucil, cytosine arabinoside, 5-fluorouracil, lomustine,

TABLE I

INTRA-ASSAY PRECISION AND ACCURACY IN PLASMA

$n = 6$.

Concentration added (μmol/l)	Concentration measured (mean ± S.D., μmol/l)	Coefficient of variation (%)	Recovery* (%)
10.0	10.21 ± 0.13	1.3	102
5.0	5.72 ± 0 09	1.6	114
2.0	1.99 ± 0.05	2.7	99
1.0	1.08 ± 0.02	2.2	108

*The accuracy of the method is expressed as the percentage recovery, calculated from the ratio of the mean concentration measured to the true value (i.e. the amount added) in plasma.

TABLE II

INTER-ASSAY PRECISION OF DETERMINATION OF I IN PLASMA

The reproducibility of the method was tested over a three-month period in fifteen consecutive assays.

Concentration added (μmol/l)	Concentration measured (mean ± S.D., μmol/l)	Coefficient of variation (%)
10	10.53 ± 0.56	5.3
5	5.98 ± 0.24	4.1
2	2.07 ± 0.07	3.6
1	1.10 ± 0.05	4.5

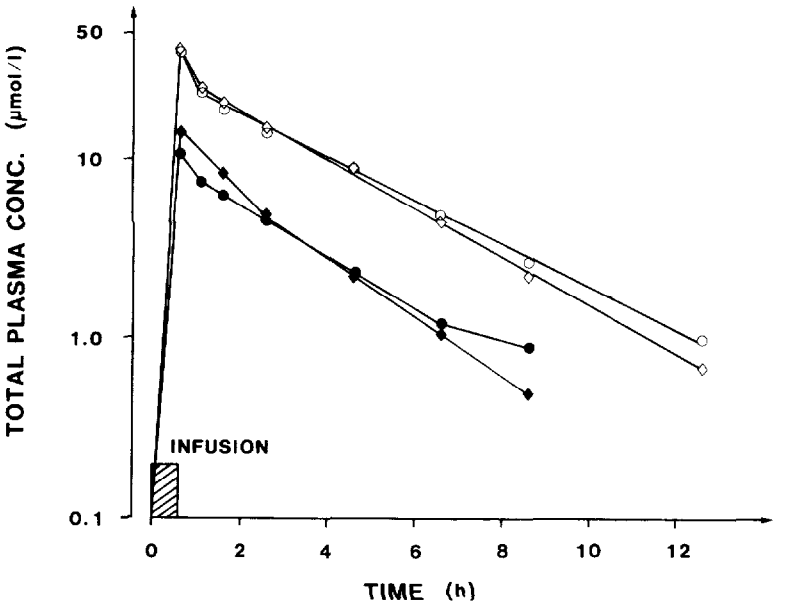

Fig. 3. Concentration—time profiles of I (open symbols) and amsacrine (closed symbols) after equimolar infusions in two rabbits. Each point is the mean of duplicate determinations.

melphalan, methotrexate, prednisolone, 6-thioguanine, vincristine and vinblastine did not interfere with the I or I.S. peaks with our extraction procedure and under these chromatographic conditions.

The assay method was applied to the measurement of I in rabbit plasma after a 35-min infusion of 12.7 μmol/kg. This dose and rate is approximately equivalent in molar terms to the amsacrine infusion received by our acute myelogenous leukaemic patients [3]. The concentration—time profile observed in two rabbits is illustrated in Fig. 3. Also included are the concentration—time profiles for an equimolar amsacrine infusion in the same rabbits.

In summary, we have extended our amsacrine assay to allow the determination of a new analogue in plasma. This method is relatively rapid and very reproducible and allows the drug to be estimated with good accuracy and precision down to a concentration of 0.1 μmol/l in 0.5 ml plasma which is sufficient for pharmacokinetic studies in patients and laboratory animals.

ACKNOWLEDGEMENT

This work was supported by the Medical Research Council of New Zealand.

REFERENCES

1 J.L. Jurlina and J.W. Paxton, J. Chromatogr., 276 (1983) 367.
2 J.W. Paxton and J.L. Jurlina, Pharmacology, in press.
3 J.L. Jurlina, A.R. Varcoe and J.W. Paxton, Cancer Chemother. Pharmacol., 14 (1985) 21.
4 G.P. Bodey and C. Jacquillat (Editors), Amsacrine, Current Perspectives and Clinical Results with a New Anticancer Agent, Proc. Int. Symp., Nice, France, August 1982, Communications Media for Education, Princeton Junction, NJ, 1983.
5 B.C. Baguley, W.A. Denny, G.J. Atwell, G.J. Finlay, G.W. Rewcastle, S.J. Twigden and W.R. Wilson, Cancer Res., 44 (1984) 3245.