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Determination of Elsamitrucin (BMY-28090) in plasma and urine by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

The cytostatic agent Elsamitrucin is a new fermentation product active in a variety of *in vivo* tumor models of murine and human origin. To determine its pharmacokinetics during the clinical phase I trial, an HPLC procedure was developed and validated. Plasma samples were extracted after addition of the internal standard, *i.e.* the analog Chartreusin. Urine samples were injected without extraction of the samples. Because of the wide concentration range of Elsamitrucin in the plasma samples two standard curves were used: up to 100 n*M* and from 100–1000 n*M*. Recoveries of Elsamitrucin from plasma were 87% and 74% for concentrations lower and higher than 100 n*M*, respectively. The detection limits were 1 n*M* in plasma and 7.5 n*M* in urine at a signal-to-noise ratio of 3. The accuracy ranged from 95–107% for plasma and from 96–104% for urine. The within-day precision was ≤4.8% and ≤2.8% in plasma and urine, respectively. The between-day precision was ≤4.4% and ≤7.1% in plasma and urine, respectively. The method proved to be sufficiently sensitive, specific and accurate for analysis of clinical samples for pharmacokinetic purposes.

INTRODUCTION

Elsamitrucin (BMY-28090) is a novel antibiotic produced by an unidentified actinomycete strain, first described by Konishi *et al.* [1,2]. Its structure, shown in Fig. 1, is related to Chartreusin, containing the same aglycone Chartartin, but with two novel sugars, Elsarose and Elsaminose [3,4]. The aminosugar Elsaminose increases the water solubility of Elsamitrucin, which is reflected by a change in the pharmacokinetics, such as a lower biliary excretion and a greater plasma clearance [3–5].

The compound prolongs the lifespan of mice with leukemia P388, leukemia L1210, melanoma B16 and sarcoma M5076 [5,6]. Potency has also been found in subrenal capsule assays and in human colon tumour xenograft [5,6]. Moreover, cytoxicity in murine and human tumour cell lines has been reported [5]. It is thought that the mechanism of action is the production of single

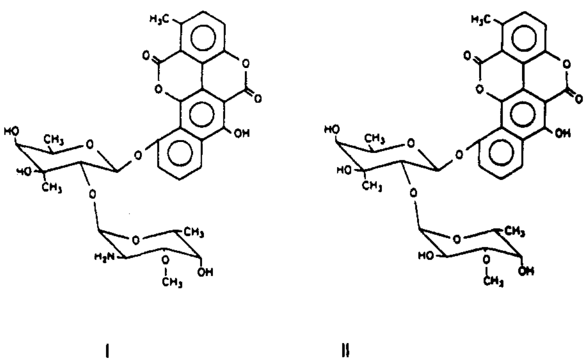
I II

Fig. 1. Structures of Elsamitrucin (I) and Chartreusin (II).

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strand breaks in DNA and/or the inhibition of the topoisomerases I and II [6,7]. Because of these findings Elsamitrucin was chosen for phase I trials [8–11], which necessitated the development of an accurate assay for the analysis of Elsamitrucin in body fluids. Therefore, we modified the existing procedure [12] by using Chartreusin, instead of 1-naphtol, as the internal standard. Chartreusin was preferred, because its structure was closely related to that of Elsamitrucin and it allowed the use of a fluorescence detector at the common emission wavelength of 455 nm.

EXPERIMENTAL

Materials

Elsamitrucin and Chartreusin were provided by Bristol-Myers Squibb, Syracuse, NY, USA. Acetonitrile, H_3PO_4 and Na_2HPO_4 were obtained from Merck (Amsterdam, Netherlands), sodium succinate from B.D.H. (Poole, UK), methanol and ethylacetate from Baker (Deventer, Netherlands), triethylamine from Pierce (Rockford, IL, USA). All reagents were of analytical grade, except ethyl acetate which was HPLC grade. Succinic acid buffer was prepared by adding 14.75 g succinic acid to 225 ml water and 10 ml 5 *M* NaOH by stirring under gentle heating until a clear solution was obtained. After cooling to room temperature, the pH was adjusted to 4.0 with 5 *M* NaOH and the volume was brought to 250 ml with water. Stock solutions of Elsamitrucin (20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 *μM* and 1000, 500, 250, 125, 62.5 and 31.25 n*M*) and Chartreusin (2.26 *μM* and 226 n*M*) were prepared in methanol.

Sample pretreatment

Plasma standard solutions were prepared by pipetting 100 *μ*l of Elsamitrucin stock solution into polypropylene tubes. Stock solution (100 *μ*l) of 2.26 *μM* Chartreusin was added to each tube with an Elsamitrucin stock solution in the *μM* range, whereas 100 *μ*l stock solution of 226 n*M* Chartreusin was added to the tubes with Elsamitrucin stock solutions in the n*M* range. After evaporating the methanol (at 30°C, under a nitrogen stream) 1 ml of blank plasma was added to each tube. The mixtures were sonicated for 5 min and left at room temperature for another 10 min. Then, 0.5 ml of 0.2 *M* sodium phosphate buffer pH 8.0 was added and after short vortex-mixing 5 ml of ethylacetate were added and the tubes were gently mixed on a Heto shaker (Wilton Woltil, De Bilt, Netherlands) for 30 min. After centrifugation for 10 min at 2000 *g*, 4.5 ml of the ethylacetate layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 30°C. The residues were reconstituted with 0.25 ml of mobile phase, vortex-mixed for 30 s, sonicated for 5 min and again vortex-mixed for 30 s. The solutions were transferred to autosampler vials and 100 *μ*l was injected into the HPLC system.

Plasma samples of patients were analyzed in polypropylene test tubes provided with 100 *μ*l Chartreusin with a concentration of either 2.26 *μM* or 226 n*M*, depending on the expected concentration in the plasma samples. If necessary (sample concentrations above 1 *μM*), plasma was diluted with water. Patient samples were processed according to the procedure described for the standards.

Unknown concentrations in plasma samples were calculated by interpolation on the appropriate calibration curve of Elsamitrucin in plasma covering the range 3.125–100 or 62.5–1000 n*M*.

Urine standards were prepared by adding 100 *μ*l of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 *μM* Elsamitrucin stock solution to aliquots of 1 ml blank urine (predose sample) from the patient. Urine standards and samples were sonicated for 5 min. Then 1.0 ml of 0.5 *M* succinate buffer pH 4.0 was added to 1 ml urine. After vortex-mixing and sonicating for 1 min followed by centrifugation at 1000 *g* for 10 min, the supernatant was transferred to autosampler vials and 100 *μ*l were injected into the HPLC system. Unknown concentrations in urine samples were calculated by interpolating on the calibration curve of Elsamitrucin in urine covering the range of 31.25–2000 n*M*.

Chromatography

The HPLC system consisted of a Pye Unicam PU 4700 autoinjector (Cambridge, UK), a Spectroflow 400 solvent delivery system (Separations, H.I. Ambacht, Netherlands) and a Philips PU 4811 computing integrator (Pye Unicam, Cam-

TABLE I

WITHIN-DAY AND BETWEEN-DAY PRECISION AND ACCURACY FOR THE DETERMINATION OF ELSAMITRUCIN IN PLASMA AND URINE

Spiked concentration (n*M*)	Within-day (*n* = 10)			Between-day (*n* = 6)		
	Determined concentration (n*M*)	C.V. (%)	Accuracy (%)	Determined concentration (n*M*)	C.V. (%)	Accuracy (%)
Plasma						
5	4.8	4.8	95.2	5.4	3.3	107.0
75	74.6	1.0	99.5	70.1	4.4	93.5
350	349.4	1.3	99.8	343.4	2.2	98.1
Urine						
75	77.6	1.2	103.5	74.0	7.1	98.7
750	721.4	2.8	96.2	741.7	2.5	98.9

bridge, UK). The system was equipped with an Alltech guard column (30 × 2.1 mm I.D.) (Zwijndrecht, Netherlands) packed with ODS-2, 20 μm and a 5-μm Chromspher C_8 column, 150 × 4.6 mm I.D. (Chrompack, Middelburg, Netherlands). The mobile phase consisted of acetonitrile–water (3:7, v/v) for plasma at a flow-rate of 2.0 ml/min and of acetonitrile–methanol–water (3:1:6, v/v/v) for urine at a flow-rate of 2.0 ml/min. H_3PO_4 (1.5 ml, 85%) and triethylamine (0.5–1.5 ml) were added to one litre of each mobile phase. An F1000 fluorescence detector (excitation wavelength 265 nm, emission wavelength 455 nm) from Merck-Hitachi (Merck, Amsterdam, Netherlands) was used.

Validation of the assay

The procedure was validated by the duplicate analysis of spiked plasma samples (5, 75 and 350 n*M*) and spiked urine samples (75 and 750 n*M*) on six days to determine the between-day precision and accuracy and by the 10-fold analysis of these samples on one day to determine the within-day precision and accuracy (Table I).

RESULTS AND DISCUSSION

Chromatograms of Elsamitrucin from plasma and urine of a representative patient who received Elsamitrucin are shown in Figs. 2 and 3. At the retention times of either Elsamitrucin or Chartreusin no interfering peaks were detected

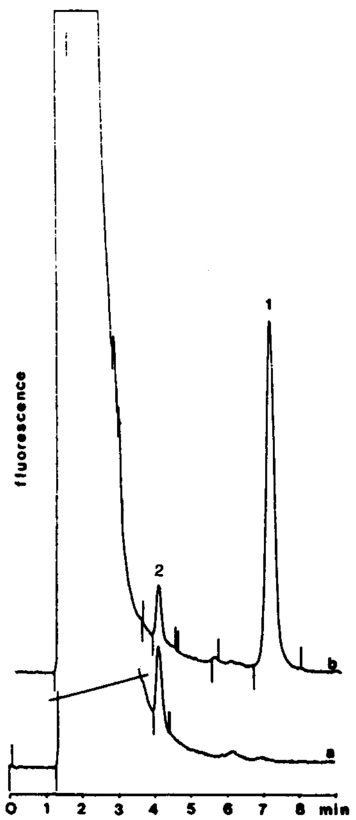

Fig. 2. Chromatograms of extracted plasma from a representative patient (a) just before and (b) 15 min after infusion. Peaks: 1 = Chartreusin (I.S., 23 n*M*), 2 = Elsamitrucin (60 n*M*).

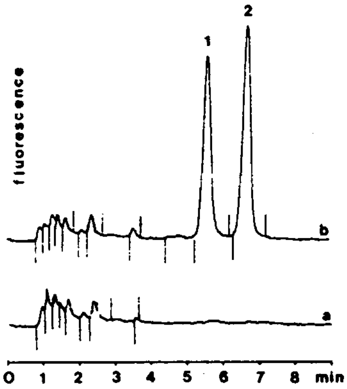

Fig. 3. Chromatograms of urine from a representative patient receiving 24 mg/m^2 Elsamitrucin: (a) blank and (b) over the first 16 h after infusion. Peak 1 = Elsamitrucin (250 n*M*).

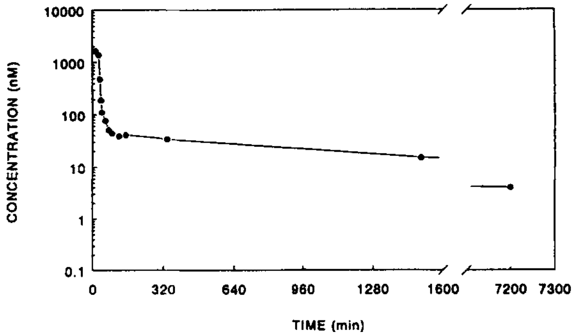

Fig. 4. A semilogarithmic plasma concentration–time curve in a representative patient after receiving 24.0 mg/m^2 of Elsamitrucin over 29 min.

in the plasmas or the urines of the patients before treatment. Calibration curves in plasma (3.125–100 n*M* and 62.5–1000 n*M*) and urine (31.25–2000 n*M*) were linear with correlation coefficients of $\geq$0.999. The detection limit of the assay (3 × noise) was 1 n*M* and 7.5 n*M* in plasma and urine, respectively. The recovery of Elsamitrucin after extraction from plasma was calculated from the ratio of the slope of the calibration curve of spiked plasma samples and the slope of the calibration curve of the same concentrations in methanol. The recovery of Elsamitrucin was 87% and 74% for the lower and higher calibration curve, respectively. The recovery of Chartreusin from plasma was *ca.* 67%. Dilution of the plasma samples with water did not change the ratios of the recoveries of Elsamitrucin and Chartreusin.

The coefficient of variation (C.V.) of the within-day repeatability ($n = 10$) was $\leq$4.8% and $\leq$2.8% in plasma and urine, respectively (Table I). The C.V. of the between-day repeatability ($n = 6$) was $\leq$4.4% and $\leq$7.1% in plasma and urine, respectively. The within-day accuracies ranged from 95–100% for plasma and from 96–104% for urine, the between-day accuracies ranged from 93–107% for plasma and were 99% for urine. As an example, Fig. 4 shows a semilogarithmic plot of the plasma concentration *versus* time curve in a representative patient who received 24 mg/m^2 of Elsamitrucin as an infusion over 29 min. This figure clearly shows that our assay allows the pharmacokinetic monitoring of Elsamitrucin for at least 5 days. We conclude that our assay of Elsamitrucin proved to be simple, accurate and precise, with a sensitivity low enough to determine the pharmacokinetics of the drug during the early stage of the clinical phase I trial.

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REFERENCES

1 M. Konishi, K. Sugawara, M. Tsunakawa, Y. Nishiyama, T. Miyaki and H. Kawaguchi, in J. Ishigami, *Proceedings of the International Congress on Chemotherapy*, Tokyo Press, Tokyo, 1985, p. 320.
2 M. Konishi, K. Sugawara, F. Kofu, Y. Nishiyama, K. Tomita, T. Miyaki and M. Kawaguchi, *J. Antibiotic.*, 39 (1986) 784–791.
3 M. Konishi and T. Oki, *Drugs Future*, 12 (1987) 1104–1105.
4 K. Sugawara, M. Tsunakawa, M. Konishi, H. Kawaguchi, B. Krishnan, H. Chun-Heng and J. Clardy, *J. Org. Chem.*, 52 (1987) 996.
5 J.E. Schurig, G.A. Basler, H.C. Faulkner, G.F. Duncan, W.C. Rose and W.T. Bradner, *Proc. Am. Assoc. Cancer Res.*, 27 (1986) 275.
6 J.E. Schurig, S. Forenza, B.H. Long, W.C. Rose, J.J. Catino, H. Kamei, Y. Nishiyama, W.T. Bradner, A.M. Casazza, D.A. Stringfellow and T.W. Doyle, *Proc. Am. Assoc. Cancer Res.*, 29 (1988) 538–539.
7 J.J. Catino, D.M. Francher, B.H. Long and A.H. Casazza, *Proc. Am. Assoc. Cancer Res.*, 28 (1987) 310.

8 R. Amato, M. Raber and L.P. Schachter, *Proc. Am. Assoc. Cancer Res.*, 30 (1989) 288.
9 B.C. Tanis, J.B. Vermorken, W.W. ten Bokkel-Huinink, W.J.F. vd Vijgh, I. Klein, C. McDaniel and H.M. Pinedo, *Proc. Am. Soc. Clin. Onc.*, 9 (1990) 71.
10 F.P. LaCreta, C. Langer, J. Walczak, P. Tinsley, L.P. Schacter, R.L. Comis, R.F. Ozols and P.J. O'Dwyer, *Proc. Am. Soc. Clin. Onc.*, 9 (1990) 72.
11 M. Raber, R. Amato, C. Sumrall and L.P. Schacter, *Proc. Am. Assoc. Cancer Res.*, 31 (1990) 178.
12 R.C. Gaver, G. Deeb and A.M. George, *Cancer Chemother. Pharmacol.*, 25 (1989) 195–201.