Journal of Chromatography, 526 (1990) 223–227
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO 5094

Note

Simplified method for the determination of atracurium and laudanosine in pig plasma by high-performance liquid chromatography and fluorimetric detection

C. SCHOPFER and A. BENAKIS*

Laboratory of Drug Metabolism, Department of Pharmacology, University of Geneva, Geneva (Switzerland)

(First received February 24th, 1989, revised manuscript received October 16th, 1989)

Atracurium besylate, 2,2'-(3,11-dioxo-4,10-dioxatridecylene)-bis-[6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinolinium] dibenzene sulphonate, is a potent neuromuscular blocking agent used in anesthesiology [1,2]. At "physiological pH", atracurium undergoes rapid Hoffmann elimination [3] to give laudanosine, which is further metabolized to tetrahydropapaverine. Laudanosine, which is metabolized by the liver and excreted by the kidneys, has been associated with convulsive seizures in animals [4].

A previous described high-performance liquid chromatographic (HPLC) method for atracurium [5] requires the use of gradient elution and the maintenance of the column at 60°C, conditions likely to reduce the column's stability and shorten its life.

To facilitate pharmacokinetic studies in pigs we were interested in developing a simplified method for the quantification of atracurium and its main metabolite laudanosine in plasma after continuous infusion of 120 μg/kg atracurium per h. We decided to use a strong cation-exchange column kept at room temperature (22 ± 0.5°C) and isocratic elution.

0378-4347/90/$03.50

EXPERIMENTAL

Materials

Atracurium and laudanosine were supplied by the Wellcome Foundation (Dartford, U.K.). D-Tubocurarine, acetonitrile and methanol (HPLC grade) were supplied by Fluka (Buchs, Switzerland). All other chemicals were supplied by Fluka and were of analytical grade. Water was doubly distilled in glass.

HPLC instrumentation

HPLC was carried out using a Spherisorb S 5 μm particle size CN, 250 mm $\times$ 4 mm I.D. (Knauer, Bad Homburg, F.R.G.) column linked to a Spectrofluorometer SFM 23 fluorescence detector (Kontron, Bern, Switzerland) set at 280 nm (excitation) and 230 nm (emission) and with a Rheodyne 7020 injector. The system was used at room temperature (22 ± 0.5 °C). The mobile phase was acetonitrile–6 g/l sodium sulphate (60:40, v/v) in 0.02 *M* sulphuric acid. It was used at a flow-rate of 1.5 ml/min.

Stabilization of plasma samples

Volumes of 1 ml of pig whole blood were collected into heparinized tubes and immediately centrifuged at 1000 *g* for 30 s. Then 200 μl of plasma were transferred to an Eppendorf tube containing 800 μl of 0.015 *M* sulphuric acid. The tube was quickly frozen to -70 °C in a mixture of acetone and dry ice. Samples were stored at -70 °C until analysis.

Analytical procedure

D-Tubocurarine was used as internal standard. After thawing, all samples including those used for preparation of the calibration curves were spiked with 20 μl of D-tubocurarine solution (100 μg/ml) and extracted with Bond-Elut Phenyl cartridges according to the method of Simmonds [5] without evaporating the eluate. A 50-μl volume of this solution was used for HPLC determination. Concentrations were calculated by the peak-area ratio of analytes to internal standard.

Calibration curves

Standard solutions used to establish the calibration curves were prepared with frozen pig plasma. After thawing, the plasma was spiked with known amounts of atracurium at concentrations ranging from 3 to 24 μg/ml and of laudanosine from 0.6 to 3 μg/ml. Internal standard solution (20 μl) was also added to each sample. Concentrations were calculated as described above. A five-point calibration curve was generated by plotting peak area against known drug concentration for each compound. The linearity of calibration curves was determined by linear least-squares regression analysis.

Precision and accuracy

The precision was evaluated by serial assay of eight to ten identical control samples of pig plasma spiked with known amounts of atracurium and laudanosine. Atracurium was tested at 5 and 20 μg/kg and laudanosine at 0.8 and 2 μg/kg. The mean, standard deviation and coefficient of variation (C.V.) were calculated for each concentration.

Additionally, the recovery was calculated by dividing the extracted drug peak area by the peak area obtained by injecting equivalent amounts of unextracted standard solutions.

Experiments in pigs

Pigs weighing 22–25 kg were premedicated subcutaneously with azaperon (4 mg/kg), ketamine (7.5 mg/kg) and fentanyl (2 mg/kg), and anesthetized with isoflurane (2–3% in oxygen). After tracheal intubation, the anesthesia was maintained with isoflurane (0.5% v/v, in oxygen) and fentanyl (2 mg/kg per h). A single intravenous bolus injection of atracurium (2 mg/kg) was given, followed by a 120 μg/kg infusion per h to obtain 90% twitch depression. Blood samples for atracurium and laudanosine were drawn every 15 min after the beginning of the infusion.

RESULTS AND DISCUSSION

Chromatography and specificity

Fig. 1 shows (A) a chromatogram of drug-free pig plasma and (B) a typical chromatogram of pig plasma after intravenous administration of atracurium by continuous infusion. The retention times of laudanosine, D-tubocurarine and atracurium were 2.5, 3.6 and 6.8 min, respectively. Although chromato-

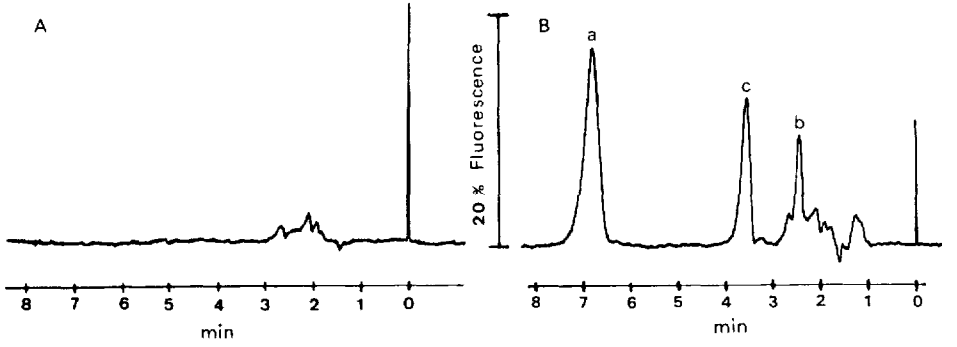

Fig. 1. (A) Chromatogram of drug-free pig plasma. (B) Chromatogram of atracurium (a) and its metabolite laudanosine (b) in pig plasma obtained 15 min after the beginning of an infusion of atracurium of 120 μg/kg per h, spiked with the internal standard D-tubocurarine (c), and extracted as described in the text.

gram B clearly shows some endogenous interference, comparison with chromatogram A demonstrates that this interference appears before the atracurium administration and is constant in time. Calculations can thus be easily made of this area so that the adjusted value of the peak can be determined. The peak in chromatogram B at 1.2 min might well be due to the presence of some other metabolites of atracurium, but since these would have been eluted before the laudanosine peak, they could not have interfered with it.

Calibration curve and reproducibility

The coefficients of correlation were 0.9997 for atracurium and 0.9982 for laudanosine, and the detection limits at a signal-to-noise ratio of 2 were 0.5 μg/ml for atracurium and 0.2 μg/ml for laudanosine. The C.V.s were calculated for each series of assays and are shown in Table I. They were less than 3.5% in all cases (range 0.54–3.44%). The recovery of analytes from plasma, estimated

TABLE I

ACCURACY AND PRECISION IN THE DETERMINATION OF ATRACURIUM AND LAUDANOSINE

Compound	Added (μg/ml)	Recovery (%)	C.V. (%)	*n*
Atracurium	5	96	3 23	10
	20	101	0.54	10
Laudanosine	0 8	95	3.44	8
	2	97	2.35	9

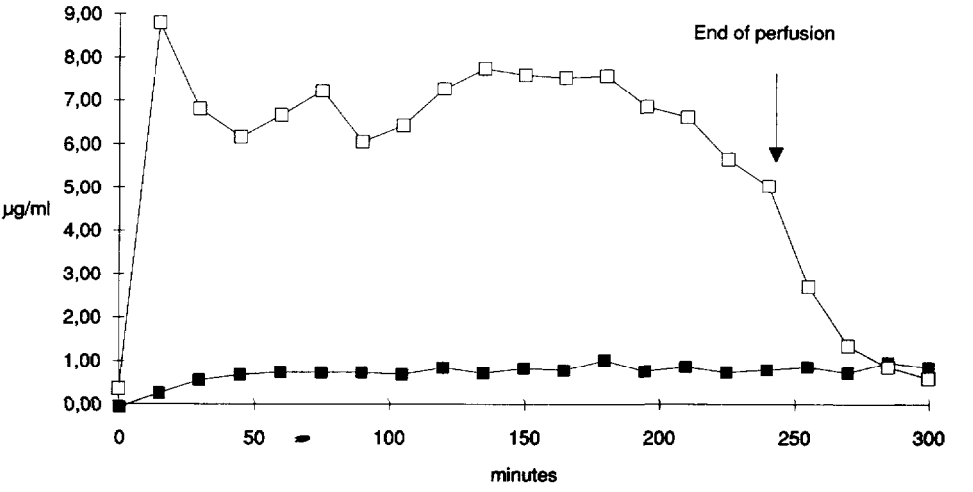

Fig. 2. Plasma concentration-time profiles of atracurium (□) and laudanosine (■) during and after atracurium infusion at 120 μg/kg per h in the pig (means of three animals).

by comparing peak areas given by unextracted standards and standards extracted from control plasma with Bond-Elut Phenyl cartridges, was at least 95% in all cases for the two substances. Recoveries are shown in Table I.

Application to biological samples

The method was applied to the determination of concentrations of atracurium and laudanosine after a continuous intravenous infusion of atracurium at a rate of 120 μg/kg per h in pigs: Fig. 2 shows the plasma concentration versus time profile of atracurium and laudanosine.

REFERENCES

1 S.J. Basta, H.H. Ali, J.J. Savarese, N. Sunder, M. Gionfriddo, G Cloutier, C. Lineberry and A.E Cato, Anaesth. Analg., 61 (1982) 723–729.
2 N.G. Goudsouzian, Br. J. Anaesth., 58 (1986) 23S–28S.
3 J.B. Stenlake, R.D. Waigh, J. Urwin, G.H. Dewar and G.G. Coker, Br. J. Anaesth., 55 (1983) 3S–10S.
4 D.J. Chapple, A.A. Miller, J.B. Ward and P.L. Wheatley, Br J. Anaesth , 58 (1986) 103S–113S.
5 R.J. Simmonds, J. Chromatogr., 343 (1985) 431–436.