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Note

Determination of etomidate in human plasma by high-performance liquid chromatography

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Etomidate is a short acting imidazole hypnotic which is commonly used for induction of anaesthesia. More recently, there is increasing interest in the use of etomidate as a sedative for ventilated patients. This application, which is currently under investigation in this hospital [1, 2], has required a precise assay for plasma etomidate. A number of procedures have been described for measurement of plasma etomidate [3—6]. Each of these has disadvantages in terms of methodological complexity or instrumental requirements.

Thus, a simple method was developed to monitor etomidate levels in these patients using high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

Etomidate [(R)-(+)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate] and propoxate hydrochloride [(+)-propyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate hydrochloride] were supplied by courtesy of Janssen Pharmaceutica (Beerse, Belgium). The solvents acetonitrile, methanol and water were HPLC grade from BDH (Poole, Great Britain). The pentane (BDH) was analytical grade further purified with activated charcoal.

Instrumentation

A Perkin-Elmer series IIIB pump solvent delivery system was used in conjunction with a Perkin-Elmer LC75 variable-wavelength ultraviolet detector (Perkin-Elmer, Beaconsfield, Great Britain). The sample was injected via a 7105 sample injector maximum loading 175 μl (Rheodyne, Berkeley, CA,

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U.S.A.). The octyl (C_8) 5-μm chromatographic column, 25 cm × 4.6 mm I.D., was manufactured by Altex Scientific (U.S.A.) and obtained from Anachem (Luton, Great Britain). A Smith Venture Mk II digital integrator was connected directly to the ultraviolet detector. Peaks were recorded on a 10-mV recorder.

Preparation of standard solutions

A stock standard solution of etomidate (1 mg/ml) is prepared in methanol. A stock internal standard solution of propoxate hydrochloride (1 mg/ml) is similarly prepared. The stock solutions are stable for more than 12 months at 4°C. A working standard solution of etomidate is prepared by diluting the stock solution 1 in 500 with water (2 μg/ml) and a working solution of propoxate hydrochloride by diluting the stock solution 1 in 250 with water (4 μg/ml). The working standards are prepared fresh for each set of assays; but they may be kept for at least three days at 4°C.

Procedure

A 10-ml volume of blood is collected into a heparinised tube containing 10 μl of saturated potassium fluoride to inhibit esterase activity [7]. The plasma is separated by centrifugation and stored at —20°C prior to assay. No significant change in plasma etomidate concentration has been detected over a 12-month period under such conditions. Add 200 μl of working internal standard to 2 ml of plasma sample, extract with 10 ml of pentane by vortex-mixing for 30 sec. Centrifuge at 1500 *g* for 5 min, then transfer the upper organic layer to a 10-ml conical centrifuge tube. Evaporate to dryness at 40—45°C in a water bath, and dissolve the residue in 200 μl of mobile phase consisting of acetonitrile—methanol—water (35:32.5:32.5).

Inject 75 μl of the aliquot onto the column and elute with degassed mobile phase at a flow-rate of 1.2 ml/min giving a pressure of 14 MPa (2000 p.s.i.). A calibration curve is prepared by adding 50, 100, 200, 400 and 500 μl of working etomidate standard to 2-ml aliquots of horse serum, corresponding to etomidate concentrations of 50, 100, 200, 400 and 500 ng/ml. These tubes are then treated as per plasma samples.

The chromatogram was recorded at 248 nm on a 10-mV recorder at 0.02 a.u.f.s. and 10 mm/min chart speed. The etomidate to propoxate peak height ratios are calculated, and these ratios are used for construction of the calibration curve and calculation of plasma etomidate concentrations.

RESULTS

Figs. 1 and 2 show typical chromatograms from a patient before and 6 min after commencement of etomidate infusion.

Recovery and linearity

Extraction efficiency was determined by comparing a standard curve for etomidate using propoxate as external standard with a standard curve using propoxate as internal standard. Within the range 0—50 ng/ml the mean

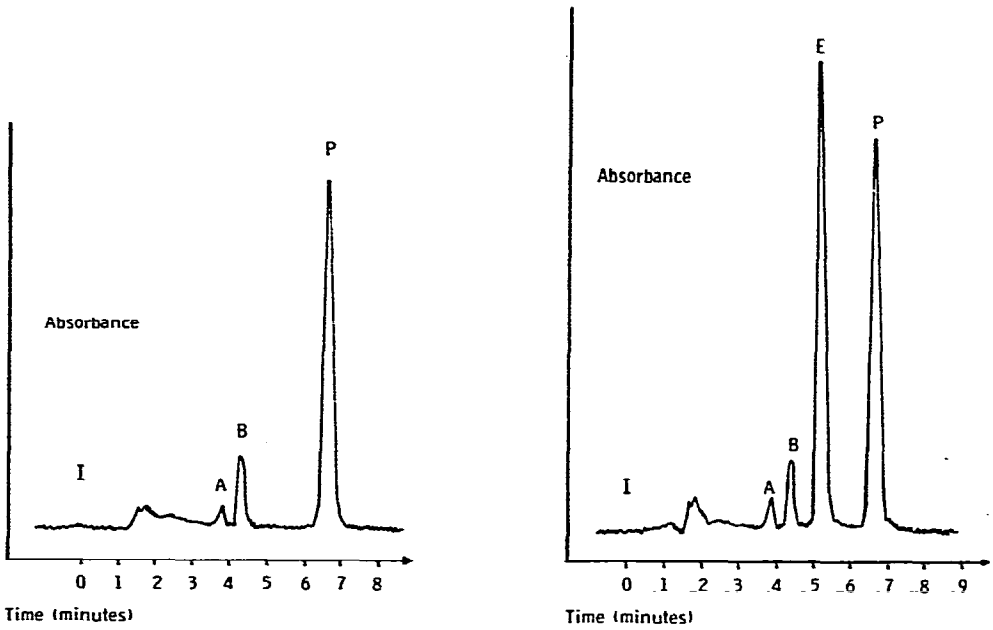

Fig. 1. Chromatogram of plasma before etomidate infusion. Peaks: P = propoxate; I = point of injection; A and B = unknown. Etomidate not detected.

Fig. 2. Chromatogram of plasma 6 min after the beginning of etomidate infusion. Bolus dose 0.3 mg/kg followed by an infusion rate of 25 μg/kg/min. Peaks: E = etomidate; P = propoxate; I = point of injection; A and B = unknown. Etomidate concentration, 306 ng/ml.

recovery was 65%, and 60% within the range 50—500 ng/ml. The standard curve was linear over the range 0—500 ng/ml.

Precision

The precision of the method was determined by calculating the coefficient of variation (C.V.) at two levels of etomidate concentration. The C.V. was 8.3% (n = 10) for 50 ng/ml and 2.8% (n = 10) for 500 ng/ml. Repeated standard curves gave similar etomidate:propoxate ratios.

Drug interference

The following drugs which have been used in conjunction with etomidate in our clinical studies were found not to interfere with the method in vitro: fentanyl, dopamine, frusemide, cimetidine and diazepam.

DISCUSSION

A number of methods have been described for the estimation of plasma etomidate. The first method, Wynants et al. [3], used gas—liquid chromatography (GLC) with a nitrogen detector. A laborious extraction technique was involved, and to achieve reasonable detection limits very pure solvents were required.

An alternative GLC method was described by De Boer et al. [5], using an

open capillary column, solid injection technique and a nitrogen detector. This used a simple extraction procedure and gave good detection limits and precision. The authors stress that a solid injection system is necessary to prevent rapid column deterioration. The short retention times (2 min for both etomidate and propoxate) would enable rapid throughput of samples but no reference is made as to whether the method is subject to drug interference.

Van Hamme et al. [4], described a mass fragmentographic method for plasma etomidate which gave excellent detection limits and precision. However, the complexity of the equipment probably precludes its use as a routine procedure.

An HPLC method has been described [6] utilising a silica gel column. However, we have been unable to obtain either the separation or sensitivity claimed by these authors.

The method described in the present paper is suitable for any laboratory possessing basic HPLC equipment. The simple extraction technique and retention times for etomidate and propoxate of approximately 5 and 7 min, respectively, allows a throughput of 15—20 samples per day.

The lack of interference in the measurement of plasma etomidate by endogenous substances is evident from the flat baseline illustrated in a patient prior to etomidate infusion (Fig. 1). It is impractical to check for interference from all drugs which could potentially be used in conjunction with etomidate, but no interference has been found by those drugs in common use in our intensive care unit. These studies were carried out by adding the drug to plasma samples in vitro. This will not detect potential inteference by drug metabolites in vivo. However, samples taken from patients receiving these drugs have not demonstrated any detectable interference, although some unidentified peaks have appeared in the chromatogram (Fig. 2).

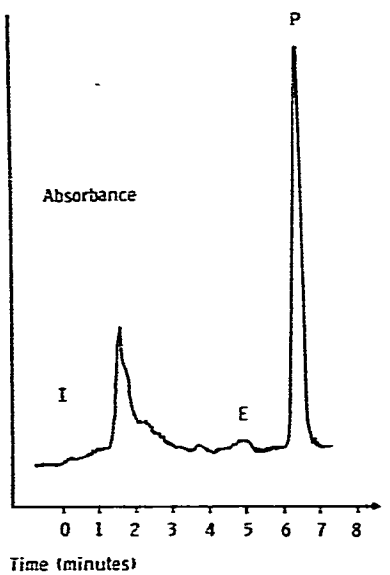

Fig. 3. Chromatogram of plasma 22 h after stopping etomidate infusion, 4 ml of plasma was extracted. Peaks: E = etomidate; P = propoxate; I = point of injection. Etomidate concentration, 4 ng/ml.

With the standard procedure as described, the detection limit is about 8 ng/ml etomidate in plasma. This detection limit may be lowered by increasing plasma volume, by further extraction of the plasma with pentane, and by increasing the percentage of extract which is applied to the column. For instance by using 4 ml of plasma and by applying 150 μl of extract to the column a detection limit of 2 ng/ml may be achieved. In our application of the method 4-ml plasma samples were used where etomidate concentrations were expected to be less than 50 ng/ml. An example of such a chromatogram with an etomidate concentration of 4 ng/ml is shown in Fig. 3.

Over the last year approximately 400 patient samples have been assayed and the method has proved suitable for routine application in the laboratory.

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