Journal of Chromatography, 529 (1990) 449–454
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5326

Note

Determination of alcuronium in plasma and urine by high-performance liquid chromatography

F. deBROS*

H.K. Beecher Laboratory, Department of Anesthesia, Massachusetts General Hospital and Harvard Medical School, Fruit Street, Boston, MA 02114 (U.S.A.)

R. OKUTANI

Teikо University, Chiba (Japan)

and

E. INADA and K. LAWRENCE

H.K Beecher Laboratory, Department of Anesthesia, Massachusetts General Hospital and Harvard Medical School., Boston, MA 0.2114 (U S.A)

(First received November 21st, 1989; revised manuscript received March 21st, 1990)

Alcuronium chloride (Alloferin® or diallyl-bisnortoxiferine dichloride) is a muscle relaxant used worldwide since it was first described in 1963 [1]. It is often cited as a reference in the characterization of new muscle relaxants [2]. Knowledge of the pharmacokinetic and pharmacologic properties of alcuronium with modern analytical techniques may be important for comparison with the new muscle relaxants.

Alcuronium (Fig. 1) is a large non-volatile molecule (MW = 2408) with a UV absorption coefficient (E) in methanol of 43 000 at 292 nm. It is stable in solution but decays in UV light. Early techniques to determine alcuronium in plasma used bioassays [3], isotope labeling [4], spectrophotometry [5] and thin-layer chromatography with a modified acid–dye reaction [6]. More recently, high-performance liquid chromatography (HPLC) [7] has been used for alcuronium quantitation after extraction from plasma with a liquid–liquid ion-pairing method. Although these techniques are sensitive, they are cumbersome to perform, lack specificity for metabolites and are rarely versatile enough to test tissue or urine over a broad range.

0378-4347/90/$03.50

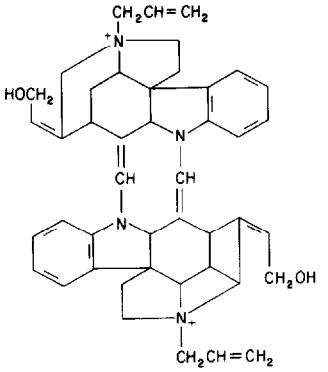

Fig. 1. Chemical structure of alcuronium. The formula is $[C_{44}H_{50}N_4O_2]^{2+}$.

We present an analytical method for alcuronium employing standard equipment and strict quality control. This new technique can measure plasma and urinary alcuronium in a broad concentration range.

EXPERIMENTAL

Reagents and solvents

Alcuronium (RO 4-3816/7) was provided by Hoffman La Roche (Nutley, NJ, U.S.A.), and *d*-tubocurarine was obtained from E.R. Squibb and Sons (Princeton, NJ, U.S.A.). Triply distilled methanol was purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Sodium lauryl sulfate was obtained form J.T. Baker (Phillipsburg, NJ, U.S.A.). Distilled, deionized, neutral charcoal-filtered water was used for all solutions. All other chemicals were of analytical grade or better.

Apparatus

A Waters (Waters Assoc., Milford, MA, US.A.) M45 solvent-delivery pump was used. The mobile phase was continuously filtered through the solvent inlet by a 3-μm filter (Waters No. 25531). Further particulate matter was removed by 2-μm and 0.5-μm filters placed ahead of the injection ports. Samples were injected through a 47-MPa six-port Valco valve with a 50-ml loop using a Glenco sample injection syringe (VIS 50-700). Separations were done on a 250 mm $\times$ 4.6 mm I.D. Waters C_{18} column (10-μm particles) at ambient temperature. The concentration of drug in the column effluent was monitored by a Waters UV detector (Model 440). Chromatograms were recorded on a Model 252A Linear Instruments strip chart recorder at a speed of 1 cm per 6 min.

Chromatographic conditions

Separations were performed at 1.4 ml/min and a back-pressure of approximately 13.5 MPa. The effluent was monitored at 280 nm with an absorbance range between 0.02 and 2.0 a.u.f.s.

Mobile phase

The aqueous component was prepared by dissolving 1.44 g of sodium lauryl sulfate in 1000 ml of water and adding 2.5 ml of glacial acetic acid. The final mobile phase was prepared in batches for daily determinations, by mixing the aqueous component (20%) with methanol (80%).

Extraction procedure, materials and method

Bond Elut™ columns (100 mg) from Analytichem International (Harbor City, CA, U.S.A.), were used with the Vac Elut™ extraction manifold.

The extraction solution consisted of 12 g of NaH_2PO_4 plus 1.2 ml concentrated H_3PO_4 per 100 ml water mixed with acetonitrile in a 1:1 ratio.

Alcuronium and *d*-tubocurarine (the internal standard) were extracted as follows. First the 100-mg Bond Elut column was prepared by a sequential wash with 2 ml water and 1 ml methanol. Then, the 1-ml plasma sample (or the 10-ml urine sample) was added, followed by 2 ml water and 1 ml water plus acetonitrile (50%). The drugs were eluted with 300 μl of the extraction solution. The extracted samples were injected onto the column immediately and remnants were stored at $-70\,^{\circ}C$ for later analysis and stability measurements.

Stock solutions

The alcuronium standard was prepared by dissolving 50 mg in 100 ml of deionized water. This solution was used for preparation of standard curves and absolute recovery studies. The internal standard was prepared by dissolving 3 mg of *d*-tubocurarine in 100 ml of deionized water. All solutions were stored at $4\,^{\circ}C$ in the dark.

Standard curves

Plasma standard curve samples were prepared as follows. Plasma was separated from blood by centrifugation. Then, 1500 ng *d*-tubocurarine (50 μl of the stock solution) and 50–5000 ng alcuronium were added to 1 ml plasma. For urine standard curves, similar amounts were added to 10-ml sample volumes.

Study of blood concentrations

In a 60-kg patient who had received 12 mg alcuronium intravenously as a "bolus", blood samples were drawn at various intervals over a 5-h period. The plasma was immediately separated and the samples were frozen at $-70\,^{\circ}C$. On the day of analysis, the samples were thawed after which the internal standard was added. All other procedures followed the protocol. A four-point standard

curve (0, 50, 500 and 5000 ng alcuronium) was used for calibration, and samples were measured in duplicate.

Quantitation

The procedure was standardized by analyzing drug-free plasma or urine samples spiked with known quantities of alcuronium chloride solution. Peak-height ratios of alcuronium chloride versus *d*-tubocurarine were used to establish calibration curves.

Reproducibility

Within-assay reproducibility was determined by performing replicate analysis of spiked plasma and urine samples.

RESULTS

Fig. 2B and C show retention times for alcuronium and *d*-tubocurarine to be approximately 7 and 10 min, respectively. Fig. 2A and D show chromatograms of drug-free samples. The standard curve for 97 plasma samples was linear with a regression factor of 0.986, a slope of 0.0015 and an intercept of −0.0044. The inter-assay coefficient of variation (C.V.) was less than 7.22%. For urine samples, the standard curve was linear with an intercept of −0.0002, a slope of 0.0023, a regression factor of 0.9998 and an inter-assay C.V. of 7.3% for 29

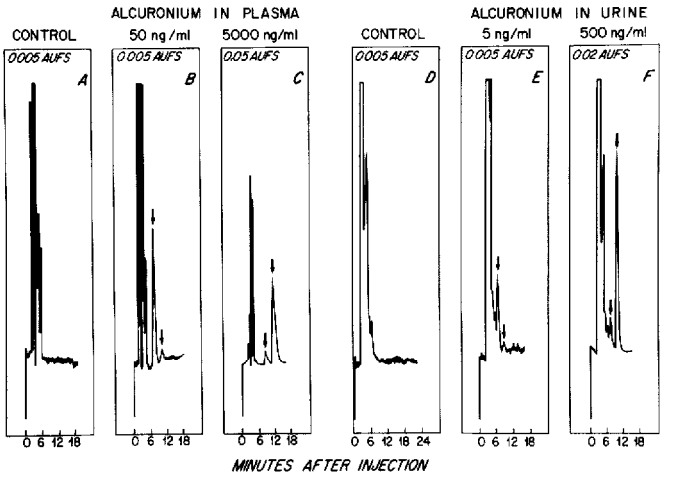

Fig. 2. Chromatograms of plasma and urine spiked with alcuronium. Baseline returns to control values 3 min after the void peak has started, *d*-tubocurarine (1500 ng) elutes at 7.2 min, alcuronium at 10.4 min, with a return to baseline inbetween. Chromatograms A and D are from drug-free samples. Chromatograms B and E are at the low end and C and F at the high end of the standard curve.

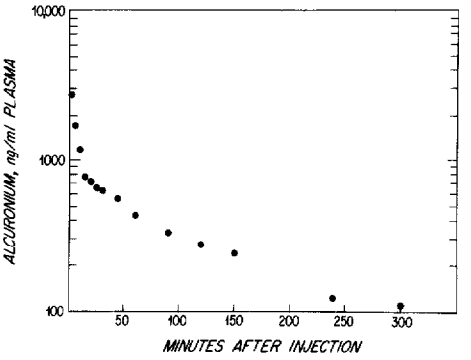

Fig. 3. Plasma decay of alcuronium in a patient having received 0.2 mg/kg intravenously at time 0. The apparent terminal half-life is 200 min, and pharmacologic effects are no more evident after 100 min.

samples. The intra-assay C.V. was less than 2% for plasma and urine. Fig. 3 shows the fate of alcuronium in plasma in a patient who received 0.2 mg/kg intravenously.

The recoveries of the drugs were calculated by comparing total sample peak height with the peak height of a series of known amounts. Recoveries for alcuronium were 92% in plasma and 88% in urine. Recoveries for *d*-tubocurarine were 79% in plasma and 75% in urine.

DISCUSSION

Our retention times for alcuronium and *d*-tubocurarine were in excellent correlation with those of Parkin [7] and confirm the reliability of the separation technique. Fig. 2A and D show no interference in drug-free samples. No drugs used for general anesthesia seemed to interfere although we did not systematically test for all possibilities. However, unlike Parkin [7] we found no impurities or "minor metabolites" at either low or high plasma concentrations of alcuronium. Perhaps the picric acid–picrate ion used by Parkin [7] slightly alters the ion-exchange characteristics of the bonded stationary phase during separations and therefore might interfere with the chromatography of alcuronium as it does with *d*-tubocurarine and its isomer, *d*-isochondodendrocurine [8]. We tend to agree with Von Raaflaub [4] that alcuronium is excreted unmetabolized.

It was noted that, as in all assays for charged drugs in urine, there is more variability at low concentrations, as highly charged molecules eluting during solid-phase extraction compete for, or "crowd out" sites at which the drugs of interest bind. If increased accuracy at low concentrations in urine (below 50 ng/ml) is desired, it is advisable to calibrate the system with a second, low-

range standard curve. In daily practice, we found that a three-point standard curve for each patient, either for urine or for plasma samples, was adequate.

The standard curves are linear over the entire therapeutic range and useful determinations can be done from 50 to 5000 ng/ml in plasma and from 5 to 500 ng/ml in urine.

The data presented on the fate of alcuronium in plasma (Fig. 3) correspond well with those of others [5]. Our data and other pharmacodynamic studies [7] suggest that concentrations of alcuronium below 300 ng/ml of plasma do not result in any significant amount of neuromuscular block and that the terminal half-life of alcuronium in plasma is approximately 200 min.

CONCLUSION

We present a versatile and universally applicable technique to measure alcuronium in plasma and urine. This assay is simple, sensitive, accurate, rapid and economical. It is applicable for clinical, pharmaceutical and forensic purposes.

REFERENCES

1 A. Boller, H. Els and A. Furst, U.S. Pat., 3 080 373 (1963); Bernauer et al., Helv. Chem. Acta, 41 (1958) 2296–2297.
2 C.A. Shanks, Anesthesiology, 64 (1986) 72–96.
3 J. Thomas, C.R. Climie and L.E. Mather, Br. J. Anaesth., 41 (1969) 297–302.
4 J. von Raaflaub and P. Frey, Arzneim.-Forsch., 22 (1972) 73–78.
5 J.S. Walker, C.A. Shanks and K.F. Brown, Clin. Pharmacol. Ther., 33 (1983) 510–516.
6 J.S. Walker, E.J. Triggs and C.A. Shanks, Eur. J. Clin. Pharmacol., 17 (1980) 449–457.
7 F.E. Parkin, J. Chromatogr., 225 (1981) 240–244.
8 F. deBros and A. Gissen, Anesthesiology, 5 (1979) S265.