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Note

Quantitation of D-tubocurarine in human plasma using high-performance liquid chromatography

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D-Tubocurarine is a natural non-depolarizing neuromuscular blocking agent largely used in anesthetic practice. Pharmacological data indicate a neuromuscular blockage with very low serum concentrations [1, 2].

Several methods have been used to assay D-tubocurarine in biological fluids in order to determine its pharmacokinetic and pharmacodynamic properties in man. D-Tubocurarine plasma concentrations have been determined by radioimmunoassay [3], fluorimetry after formation of a complex with a dye [4, 5], and more recently by high-performance liquid chromatography (HPLC) [6, 7]; however, the latter techniques have never been applied to clinical situations.

This report describes a new method for the selective quantitation of nanogram amounts of D-tubocurarine in human plasma using ion-pair extraction and reversed-phase liquid chromatography. Preliminary results concerning the pharmacokinetics of D-tubocurarine in man using this assay are also given.

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MATERIALS AND METHODS

Chemicals and glassware

D-Tubocurarine was purchased from Bruneau, Boulogne sur Seine, France. ORG NC 45 is a gift from Organon, Oss, The Netherlands. Alloferin was purchased from Roche SA, Neuilly sur Seine, France. Methanol and 1,2-dichloroethane were of analytical grade quality and purchased from Merck (Darmstadt, G.F.R.). Phosphoric acid, hydrochloric acid and triethylamine were of analytical grade quality and purchased from Merck. Pentanesulfonic acid was purchased from Waters Assoc. as Pic B5 (Waters, Paris, France).

The glycine stock solution contained 750 mg of glycine and 585 mg of sodium chloride per 100 ml of water.

The potassium iodide—glycine buffer solution [4] was prepared freshly each day of analysis by mixing 4 ml of 0.1 *N* sodium hydroxide, 6 ml of glycine stock solution and 12.8 g of potassium iodide.

All determinations were done in polypropylene tubes.

Sample processing

To 1 ml of plasma or standard in a polypropylene tube was added 0.1 ml of glycine buffer. The contents were mixed for 2 min on a Vortex mixer, then 1 ml of ethylene dichloride was added and mixed for 30 min on a mechanical rotary-type shaker. This mixture was centrifuged at 1000 *g* for 10 min (4°C); then exactly 750 μl of the ethylene dichloride layer were added to a second conical polypropylene tube (Beckman) with 200 μl of 0.01 *N* hydrochloric acid. The contents of this second test tube were mixed by swirling for 30 sec and centrifuged for 2 min. Exactly 120 μl of the aqueous phase were pipetted for injection into the chromatograph.

HPLC apparatus and phase system

The HPLC set-up consisted of a Waters Model 6000A pump, a Waters U6K universal injector and an UV detector Model 440 (Waters). All chromatograms were obtained using a linear potentiometric recorder (one channel) (Kipp & Zonen).

Throughout the investigation a radial compression system with a μC_{18} column (particle size 10 μm, 15 cm × 8 mm I.D.; Rad-Pack A, Waters) used in an isocratic mode. The UV detector was set at 280 nm with the appropriate filter on 0.005 absorbance unit. The mobile phase consisted of 40% of methanol in an aqueous mixture of triethylamine (10 g/l), Pic B5 (1 ml), phosphoric acid (2 ml) and distilled water to 1 liter. The final pH of the aqeuous mixture was 3.4. The eluent was filtered through a Millipore filter (0.2 μm) prior to use. The eluent flow-rate was 2 ml/min, and the recorder was 1 cm/min on 10 mV.

RESULTS

Using the described conditions D-tubocurarine has a retention time of approximately 4.4 min ($k' = 1.75$). Fig. 1 shows chromatograms of a plasma sample with D-tubocurarine and a blank plasma.

The concentration of D-tubocurarine standard and the peak height were linearly related over the range 25—500 ng/ml. The standards were obtained by dissolution of D-tubocurarine in a plasma blank.

The limit of sensitivity for D-tubocurarine was 0.025 μg/ml. The reproducibility was in a range of 6—8% within the standard curve (0.025—0.5 μg/ml). The recovery, tested by adding D-tubocurarine to known samples, was never below 95%. The linearity, tested by dilution of plasma samples, was above 97%.

Other non-depolarizing muscle relaxants were tested in this chromatographic system and showed very different retention times (NC 45, $k' = 5$; alloferine, $k' = 5.68$).

The evolution of the plasma concentration of D-tubocurarine was followed after a single intravenous injection in three patients undergoing abdominal surgery (Fig. 2).

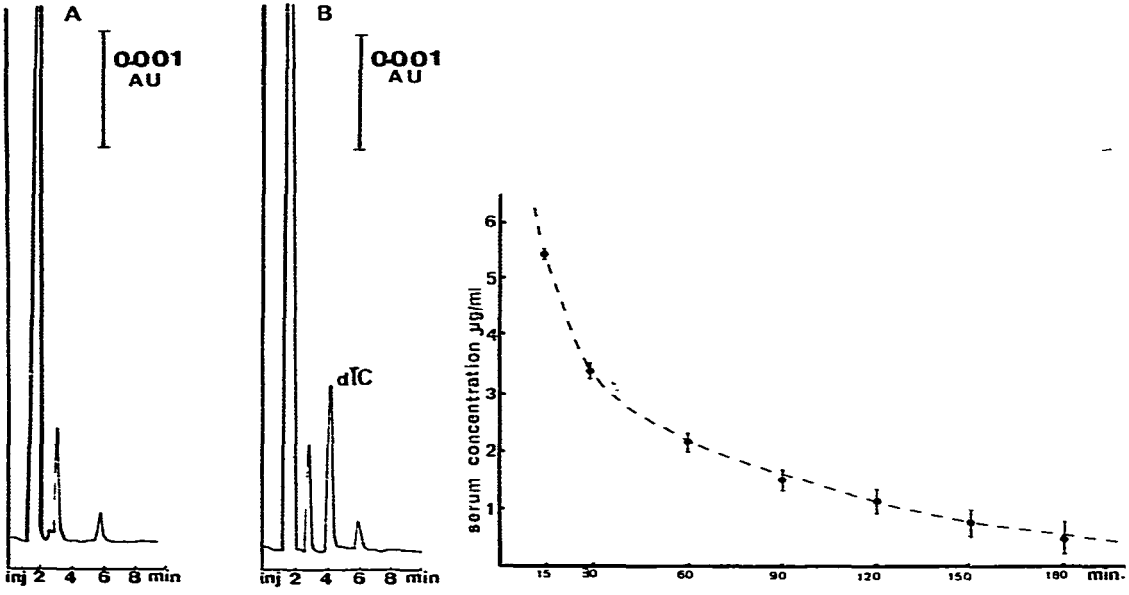

Fig. 1. Chromatograms of blank human plasma (A) and a human plasma sample from a clinical study (B). D-Tubocurarine (dTC) concentration is 95 ng/ml.

Fig. 2. Profile of D-tubocurarine plasma concentration vs. time after an intravenous bolus dose of 0.3 mg/kg. Each point is the mean of the plasma concentration of three patients. The plasma must be diluted with distilled water (1:10) for such elevated values.

DISCUSSION

Firstly, the extraction of D-tubocurarine from plasma is very important in this procedure. This two-step extraction was established by Cohen [4]. The first ethylene dichloride extraction gives a constant coefficient of 85% and the second acidic extraction gives a constant coefficient of 89%. The ratio of

the solvents' volume in the two cases determined to a large extent the limit of sensitivity of the assay.

Secondly, the chromatographic eluent was chosen to shorten the duration of the procedure on the radial compression system. The chromatogram (Fig. 1) shows that one assay lasted 5 min. The reliability of the assay was not increased when we added alloferine as internal standard before the first extraction.

CONCLUSION

This procedure is a selective and sensitive chemical assay which permits the quantitation of D-tubocurarine in the plasma of patients undergoing therapy with this drug.

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