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Note

Determination of D-tubocurarine chloride or metocurine iodide in human plasma by high-performance liquid chromatography with ultraviolet detection

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D-Tubocurarine chloride [(+)-7′,12′-dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethyltubocuraranium chloride] is the prototype of the non-depolarizing (competitive) neuromuscular blocking agents (muscle relaxants) [1]. D-Tubocurarine chloride has been widely used since its introduction to the clinical practice of anesthesia in 1942 [2]. Recently the trimethylderivative of D-tubocurarine, metocurine iodide (6,6′,7′,12′-tetramethoxy-2,2,2′,2′-tetramethyltubocuraranium diiodide), has been proposed as an alternative to D-tubocurarine chloride not only because it is twice as potent but also as it causes less autonomic blockade and histamine release [3].

Early techniques for the measurement of plasma concentrations of D-tubocurarine chloride include a micro spectrophotometric method which is sensitive to 1.0 μg/ml [4] and a spectrophotofluorometric method which, though sensitive to 20 ng/ml, is fraught with technical difficulties [5]. A sensitive and specific radioimmunoassay was later developed for the measurement of plasma concentrations of D-tubocurarine chloride [6]; a modification of this technique, using the D-tubocurarine antibody which is not commercially available, is the only method for the measurement of plasma metocurine iodide concentrations [7]. Recently two high-performance liquid chromatographic (HPLC) techniques for the measurement of plasma D-tubocurarine chloride concentrations have been reported; one uses an incompletely resolved internal standard and has a sensitivity of 100 ng/ml [8] while the other has sensitivity to 25 ng/ml but as it uses no internal standard depends on very precise volume measurements [9].

It was the purpose of the present study to develop, for purposes of pharmacokinetic analysis, a simple and sensitive HPLC technique for the determination of plasma concentrations of D-tubocurarine chloride and metocurine iodide. As

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neither is metabolized [10], one could be used as the internal standard for the other. An important feature of this technique is the facile isolation of these quaternary ammonium compounds from an aqueous matrix with inexpensive disposable solid phase extraction columns.

EXPERIMENTAL

Reagents

The D-tubocurarine chloride (Calbiochem, San Diego, CA, U.S.A.) and metocurine iodide (Diosynth, Chicago, IL, U.S.A.) reference standards were used after drying at room temperature in a vacuum desiccator. The dibutylamine was organic reagent grade and the phosphoric acid was reagent grade (Mallinckrodt, Paris, KY, U.S.A.). Acetonitrile, methanol, and tetrahydrofuran were HPLC grade (Waters Assoc., Milford, MA, U.S.A.). All other reagents were analytical reagent grade.

Sample collection and preparation

Blood samples (5 ml) were obtained by syringe through a 16-gauge PTFE-catheter, previously inserted in a radial artery for blood pressure monitoring, and transferred to Vacutainer (Becton-Dickinson, Rutherford, NJ, U.S.A.) collection tubes containing sodium heparin. The plasma samples were removed after centrifugation of the blood for 10 min at 1800 *g*, transferred to polypropylene test tubes, and stored at −30°C until extracted in duplicate.

Sample extraction

Tubocurarine and metocurine were extracted from plasma using Bond-Elut 100 mg C_{18} solid phase extraction columns in conjunction with the Vac-Elut ten-place vacuum manifold (Analytichem International, Harbor City, CA, U.S.A.). The columns were conditioned by sequential flushes (under vacuum) of 2 column vol. of tetrahydrofuran, 2 vol. of methanol, and 2 vol. of water. Then 0.5-ml plasma samples (1.0 ml for the very lowest plasma concentration) were added to the columns along with 50 μl (100 μl with the 1.0 ml plasma samples) of the appropriate internal standard solution, 10 μg metocurine iodide per ml 0.01 mol/l hydrochloric acid for the D-tubocurarine chloride assay and 5 μg D-tubocurarine chloride per ml 0.01 mol/l hydrochloric acid for the metocurine iodide assay, and the vacuum reapplied. The columns were next washed with 2 vol. of water. Subsequent application of 250 μl of the HPLC mobile phase (described below), under vacuum, eluted the D-tubocurarine and metocurine from the columns into 1.5 ml-Eppendorf micro test tubes (Brinkman Instruments, Westbury, NY, U.S.A.) where they were allowed to dry. Prior to injection into the HPLC the samples were reconstituted with 100 μl of the HPLC mobile phase (described below), mixed on a vortex mixer, and centrifuged at 12,800 *g* for 5 min in the Eppendorf Micro Centrifuge Model 5412 (Brinkman Instruments).

Chromatographic apparatus and conditions

The HPLC system consisted of Waters Assoc. Model 6000A solvent delivery system, Model U6K universal liquid chromatograph injector, RCM-100 radial

compression separation system with a Radial-Pak 10-μm CN cartridge and Guard-Pak 10-μm CN precolumn insert, and Model 450 variable-wavelength detector set at 204 nm. D-Tubocurarine and metocurine were eluted isocratically at ambient temperature and at 2.4 ml/min with an acetonitrile—methanol—water—1.0 mol/l dibutylamine phosphate (pH 2.5) (40 : 10 : 10 : 1) mobile phase that had been filtered through a 0.22-μm Durapore Filter (Waters Assoc.). The chromatograms were recorded, the peaks were identified and integrated, and concentrations were reported on the basis of the internal standard area ratio method by the 3390A Reporting Integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Evaluation of the methods

The linearity, accuracy, and precision of the assays were assessed by the measurement of the D-tubocurarine chloride or the metocurine iodide concentra-

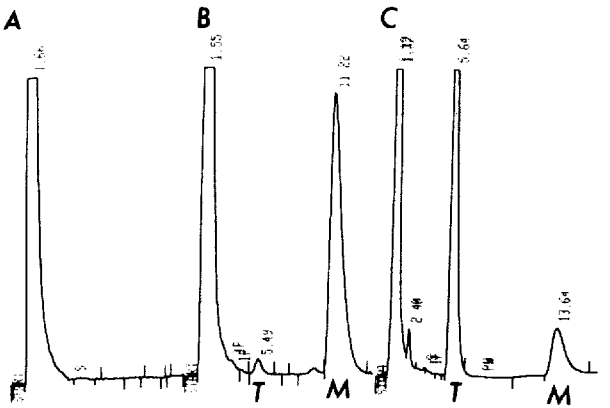

Fig. 1. Chromatograms of D-tubocurarine chloride (T) extracted with the internal standard, metocurine iodide (M), 1000 ng/ml. The ordinate is response and the abscissa is min. The retention times for the peaks are indicated on the chromatograms. A, blank plasma; B, plasma standard with 25.0 ng/ml D-tubocurarine chloride; C, plasma of a patient obtained 5 min after the administration of 0.5 mg/kg D-tubocurarine chloride.

TABLE I

ACCURACY AND PRECISION FOR THE PLASMA D-TUBOCURARINE CHLORIDE ASSAY (n = 8)

D-Tubocurarine chloride added (ng/ml)	D-Tubocurarine chloride measured (ng/ml)*	Mean error (ng/ml)	Relative error (%)	C.V. (%)
25	25.0 ± 2.0	0.0	0.0	7.9
50	50.0 ± 2.4	0.0	0.0	4.8
100	98.0 ± 4.6	2.0	2.0	4.7
250	252.8 ± 9.4	2.8	1.1	3.7
500	488.7 ± 18.7	11.3	2.3	3.8
1000	993.3 ± 41.9	6.7	0.7	4.2
2500	2488.6 ± 86.9	11.4	0.5	3.5
5000	4992.5 ± 171.7	7.5	0.2	3.4

*Mean ± S.D.

tions of replicate plasma standards over a period of several weeks. The plasma standards were made by adding known amounts of D-tubocurarine chloride or metocurine iodide from stock solutions to blank normal human plasma. These plasma standards were in the useful clinical range and contained 25.0, 50.0, 100, 250, 500, 1000, 2500, or 5000 ng/ml of either D-tubocurarine chloride or metocurine iodide. Recovery was evaluated by comparing the peak areas of 25.0, 250, and 2500 ng/ml extracted plasma standards to those of the standard stock solutions.

Clinical study

The usefulness of these methods for clinical pharmacokinetic studies was evaluated, after obtaining institutionally-approved informed consent, in two adult males scheduled to have vascular surgery. Anesthesia was induced in these patients with intravenous thiopental and intravenous succinylcholine was used

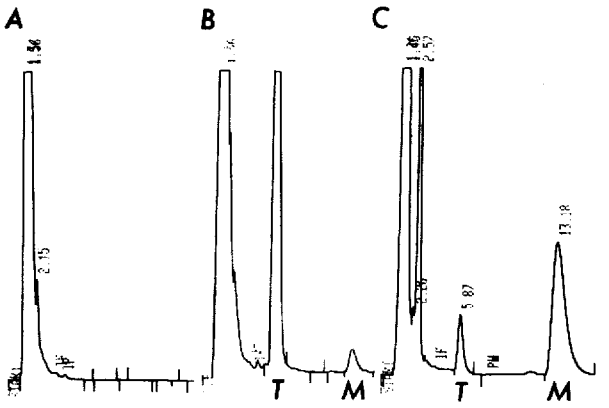

Fig. 2. Chromatograms of metocurine iodide (M) extracted with the internal standard, D-tubocurarine chloride (T), 500 ng/ml. The ordinate is response and the abscissa is min. The retention times for the peaks are indicated on the chromatograms. A, blank plasma; B, plasma standard with 25.0 ng/ml metocurine iodide; C, plasma of a patient obtained 5 min after the administration of 0.3 mg/kg metocurine iodide.

TABLE II

ACCURACY AND PRECISION FOR THE PLASMA METOCURINE IODIDE ASSAY (n = 8)

Metocurine iodide added (ng/ml)	Metocurine iodide measured (ng/ml)*	Mean error (ng/ml)	Relative error (%)	C.V. (%)
25	25.8 ± 1.7	0.8	3.2	6.5
50	48.7 ± 2.3	1.3	2.6	4.8
100	100.7 ± 4.2	0.7	0.7	4.1
250	251.5 ± 8.4	1.5	0.6	3.3
500	493.8 ± 8.5	6.2	1.2	1.7
1000	1004.8 ± 25.9	4.8	0.5	2.6
2500	2495.0 ± 79.5	5.0	0.2	3.2
5000	5000.4 ± 143.2	0.4	0.0	2.9

*Mean ± S.D.

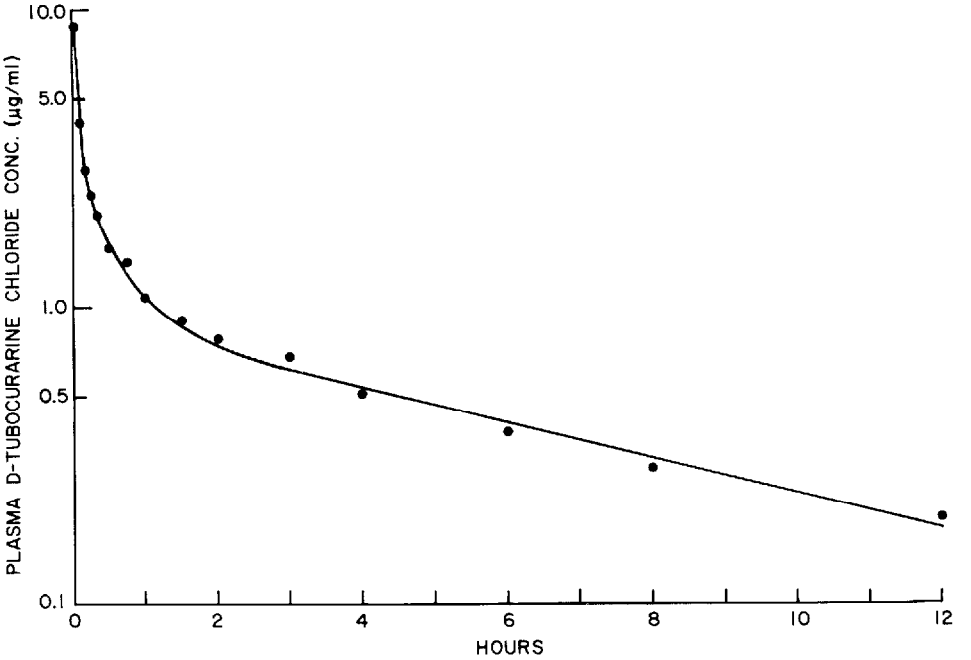

Fig. 3. Plasma D-tubocurarine chloride versus time relationship after the administration of 0.5 mg/kg to a 91-kg 68-year old male for surgical muscle relaxation. The solid line is a computer-derived non-linear least squares regression line through the actual patient points.

to facilitate tracheal intubation. Anesthesia was maintained with 70% nitrous oxide in oxygen and intravenous doses of fentanyl. Muscle relaxation was provided with a rapid (30 sec) intravenous injection of D-tubocurarine chloride, 0.5 mg/kg, or metocurine iodide, 0.3 mg/kg, and was reversed at the end of the anesthetic by intravenous atropine and neostigmine. Blood samples were obtained before and 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 480, and 720 min after the administration of either the D-tubocurarine chloride or metocurine iodide.

RESULTS AND DISCUSSION

High-performance liquid chromatograms of D-tubocurarine chloride and metocurine iodide are shown in Figs. 1 and 2, respectively. None of the drugs administered concomitantly in the clinical study were found to interfere with the assay.

The accuracy and precision of the HPLC techniques for the measurement of D-tubocurarine chloride and metocurine iodide are summarized in Tables I and II, respectively. Linear regression analyses of the standard muscle relaxant concentrations from 25.0—5000 ng/ml versus muscle relaxant : internal standard area ratios verified the linearities of both the D-tubocurarine chloride standard curve ($r = 0.999$; $y = 852x + 4$) and the metocurine iodide standard curve ($r = 0.999$; $y = 571x + 1$). The average recoveries for eight replicate samples at 25.0, 250, and 2500 ng/ml were 79.4, 82.4, and 79.0%, respectively, for

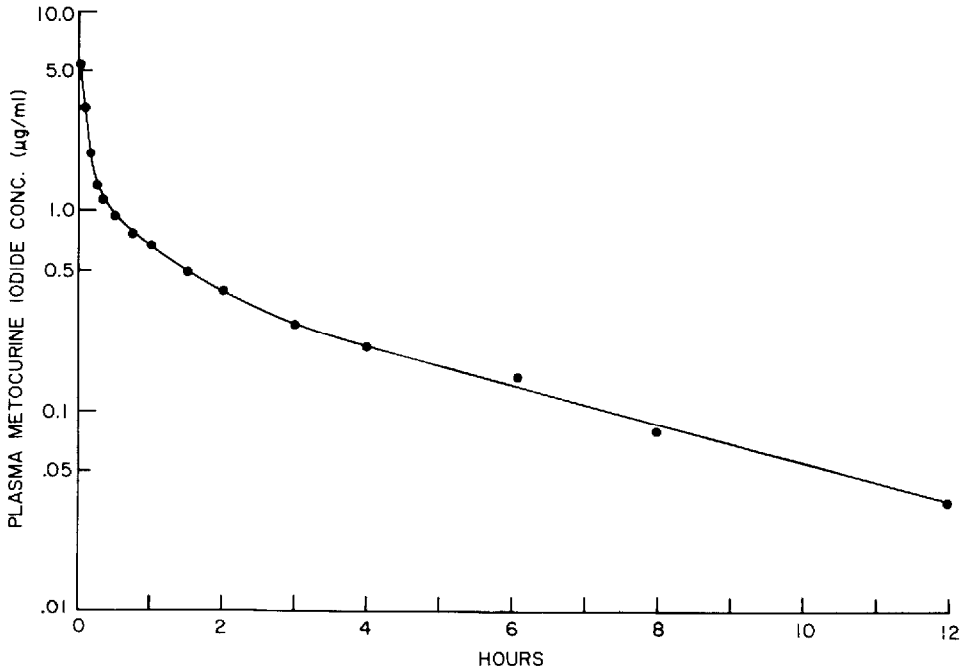

Fig. 4. Plasma metocurine iodide versus time relationship after the administration of 0.3 mg/kg to a 90-kg 50-year old male for surgical muscle relaxation. The solid line is a computer-derived non-linear least squares regression line through the actual patient points.

D-tubocurarine chloride and 78.8, 76.8, and 75.2%, respectively, for metocurine iodide.

Plasma D-tubocurarine chloride concentration and plasma metocurine iodide concentration versus time relationships in the two patients are illustrated in Figs. 3 and 4, respectively. The present HPLC techniques for the measurement of these two drugs are, therefore, able to accurately and easily measure the plasma concentrations of these two drugs for at least 12 h after the administration of the normal clinical doses.

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