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Short Communication

Rapid determination of succinylcholine in human plasma by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

A high-performance liquid chromatographic method with fluorometric detection has been developed for the determination of succinylcholine in human plasma. Succinylcholine shows fluorescence at 282 nm with an excitation at 257 nm. The assay is sensitive, reproducible and linear for concentrations ranging from 100 ng/ml to 100 μg/ml of succinylcholine. In a pilot study the plasma concentration–time curve showed a triphasic elimination, with half-lives of 0.4, 1.2 and 8 min, respectively. In a clinical setting, drugs commonly administered during anaesthesia did not interfere with the assay. This method provides a simple and time-saving alternative to existing methods.

INTRODUCTION

Succinylcholine is a depolarizing neuromuscular blocking agent with rapid onset and short duration of action. Its practical application in anaesthesiology is still widespread, although its pharmacokinetics in humans is still not well understood. Although administration of this relaxant can reliably be titrated on the observed effect, study of the plasma decay curves could give more insight in the inter-individual variability of drug disposition and action. In addition, by relating

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the drug concentration to the effects, it should be possible to learn more about the pharmacodynamic variability.

Until recently, little attention has been paid to the kinetic analysis of succinylcholine because of the lack of simple techniques to monitor plasma concentrations. In addition, pseudocholinesterase present in blood and sometimes in urine rapidly decomposes succinylcholine *in vitro*, making drug analysis difficult and meaningless. Detection of succinylcholine in pharmacological investigations has been attempted by utilizing radiolabelled drug [1,2] or indirectly by bioassay [3,4]. Other methods for the analysis of succinylcholine in biological specimens include: (1) thin-layer chromatography (TLC) on silica gel or cellulose plates [5]; (2) gas chromatography–mass spectrometry (GC–MS) based on N-demethylation by benzenethiolate to a tertiary amine with suitable GC properties [6–11]; (3) coloured lipophilic strong acid and ion-pair extraction into organic solvents [12].

The aim of this investigation was to develop a simple and rapid high-performance liquid chromatographic (HPLC) analysis of succinylcholine in human plasma.

EXPERIMENTAL

Chemicals and reagents

Solvents of HPLC grade and all other chemicals of analytical grade were purchased from Merck (Darmstadt, Germany). Succinylcholine (Curalest) was obtained from Pharmachemie (Haarlem, Netherlands). Echotiopate iodide was obtained from Wyeth Lab. (Hoofddorp, Netherlands). Blood tubes were obtained from Terumo Europe (Leuven, Belgium).

Drug standard

A stock solution of succinylcholine (1 mg/ml) was prepared by dissolving 25 mg of succinylcholine in 25 ml of acetonitrile–water (50:50, v/v). Drug plasma standards were prepared by spiking blank control plasma with an appropriate microlitre volume of working drug solution to obtain plasma standards with the following concentrations of succinylcholine: 0.1, 0.25, 0.50, 1.0, 2.5, 5, 10, 25, 50 and 100 μg/ml.

Sample preparation

For standard samples, 360 μl of drug-free plasma were spiked with 40 μl of different concentrations of succinylcholine. To 400 μl of standard or sample in an Eppendorf reaction vessel, 40 μl of trichloroacetic acid (500 g/l) were added and mixed thoroughly on a vortex-mixer. After centrifugation at 12 500 *g* for 5 min, 100 μl were injected into the column.

Instrumentation

The HPLC instrument consisted of an SP 8810 precision pump (Spectra Phys-

ics, Eindhoven, Netherlands), an N 60 injector Valco H (Chrompack, Middelburg, Netherlands), a fluorescence spectrometer (Perkin-Elmer 3000, Gouda, Netherlands), and a BD 40 recorder (Kipp Analytical, Delft, Netherlands). The column was packed with Cp-tm-Spher C_8 (8 μm particle size, 250 mm × 4.6 mm I.D.; Chrompack). The fluorescence spectra were measured in water and in the mobile phase. The succinylcholine concentration used for recording the spectra was 50 μg/ml; the slit width was 10 nm. Succinylcholine showed an excitation at 257 nm and emission at 282 nm. The mobile phase was acetonitrile–methanol–0.05 *M* potassium diphosphate buffer (pH 5.0) (35:5:60, v/v). The flow-rate was 1.2 ml/min, and the pressure *ca.* 12 MPa. The chromatographic system was operated at room temperature.

Calibration curve

The calibration curves were constructed by plotting the peak height against known concentrations of succinylcholine.

Patient study

A patient was brought under deep general anaesthesia with intubation and artificial respiration without the use of muscle relaxants. After a haemodynamically stable period of 10 min, succinylcholine (2 mg/kg) was given as an intravenous bolus. Blood samples of 5 ml were drawn from the arterial line at frequent intervals for up to 1.5 h. The blood was placed into 10-ml Venoject heparinized tubes containing 0.1 ml of 0.25% echotiopate iodide as a pseudocholinesterase inhibitor [13]. The blood samples were centrifuged, and plasma was separated and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Fig. 1 shows the fluorescence and excitation spectra of succinylcholine. The fluorescence spectrum has maxima at 282 and 570 nm following excitation at 257 nm. The excitation spectrum gives a maximum at 257 nm. The fluorescence maximum of 282 nm was used for the detection of succinylcholine because at this wavelength the intensity is 3.3 times higher than at 570 nm.

Fluorescence detection was also used in the HPLC of quaternary ammonium compounds of atracurium and laudanosine [14–16].

Fig. 2 shows three chromatograms of succinylcholine in the plasma of a patient after intravenous administration of 2 mg/kg succinylcholine. A drug-free sample, a low concentration of 0.5 μg/ml 15 min after injection, and a high concentration of 2.4 μg/ml 4.5 min after injection are shown.

Calibration curves were linear in the range from 100 ng/ml to 100 μg/ml. The limit of detection was 100 ng/ml ($r = 0.9986$). The retention time was 6.3 min, and the capacity factor (k') was 2.5.

The inter-day precision (Table I) expressed as the coefficient of variation

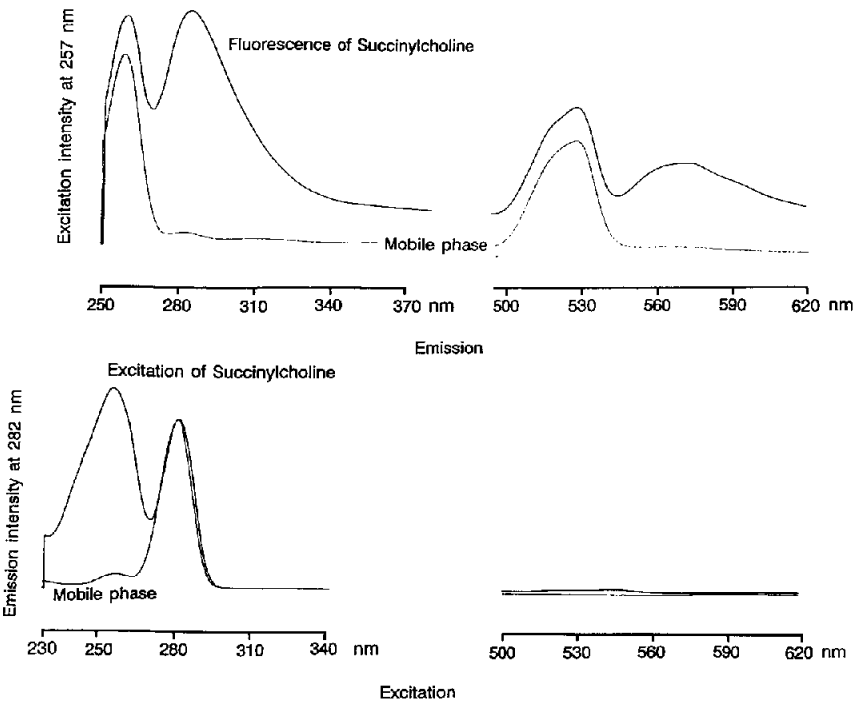

Fig. 1. Fluorescence and excitation spectra of succinylcholine in the mobile phase.

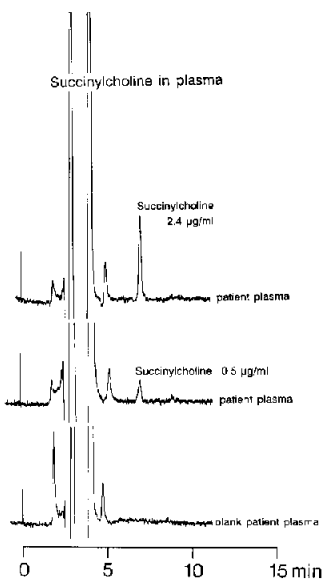

Fig. 2. Chromatograms of succinylcholine in plasma from a patient.

TABLE I

PRECISION DATA FOR THE DETERMINATION OF SUCCINYLCHOLINE IN SERUM

Concentration added (μg/ml)	n	Concentration measured (mean ± S.D.) (μg/ml)	C.V. (%)
Inter-day			
0.500 (low)	10	0.484 ± 0.041	8.5
5.000 (high)	10	5.133 ± 0.084	1.6
Intra-day			
0.500 (low)	6	0.488 ± 0.048	9.8
5.000 (high)	6	5.149 ± 0.119	2.3

(C.V.) is 8.5% at a concentration of 0.5 μg/ml and 1.6% at a concentration of 5.0 μg/ml ($n = 10$). The intra-day precision (Table I) expressed as the C.V. is 9.8% at a concentration of 0.5 μg/ml and 2.3% at a concentration of 5.0 μg/ml ($n = 6$).

Fig. 3 shows the plasma concentration–time curve of succinylcholine in a patient after intravenous administration of 2 mg/kg succinylcholine. The elimination is triphasic, with apparent half-lives of 0.4, 1.2 and 8 min.

In conclusion, this HPLC method is simple and rapid, and makes possible the pharmacokinetic analysis of succinylcholine.

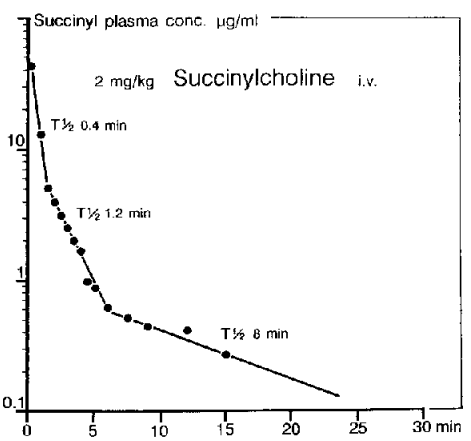

Fig. 3. Plasma concentration–time curve of succinylcholine in a patient after intravenous administration of 2 mg/kg succinylcholine.

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