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Short Communication

Simple, rapid and selective method using high-performance liquid chromatography for the determination of bretylium in plasma

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ABSTRACT

A high-performance liquid chromatographic method with ultraviolet detection has been developed for the determination of bretylium in plasma. Following a single-step solid-phase extraction procedure, bretylium is selectively isolated and well recovered from plasma. The assay sensitivity is 0.156 μg/ml from 250-μl plasma samples and its linearity was assessed up to 40 μg/ml. The method is accurate (101.0 ± 5.4%) and precise (maximum coefficient of variation of 8%). It provides a simple and time-saving alternative to existing methods and is particularly suitable for pharmacokinetic studies.

INTRODUCTION

Bretylium tosylate (2-bromo-N-ethyl-N,N-dimethylbenzenemethanaminium 4-methylbenzenesulphonate), a benzyl quaternary ammonium compound, was originally used clinically for the treatment of hypertension because of its adrenergic neuronal blocking activity [1]. Its use as an antihypertensive agent ceased because of the development of tolerance and undesirable effect during chronic oral therapy [2]. Since the antifibrillatory properties of bretylium were first described in animals [3], numerous clinical reports have appeared in the literature indicating that parenteral bretylium has significant antiarrhythmic activity, and is particularly effective in the acute control of life-threatening ventricular arrhythmias that are refractory to first-line antiarrhythmic drugs [4,5].

Despite its approval in many countries for the treatment of ventricular arrhythmias, the dosage recommendations for bretylium have been determined empirically and have yet to be optimized. Furthermore, data on the body disposition of bretylium are very limited owing to the lack of an analytical method that lends itself to pharmacokinetic analysis when numerous plasma samples have to be processed.

To date, reported methods for the determination of bretylium in plasma require derivatization

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prior to quantitation by gas chromatography (GC) coupled to flame ionization [6] or electron-capture detection [7,8]. The extraction procedure described by Kuntzman *et al.* [6] for bretylium was considerably simplified by Patterson *et al.* [7] but still requires two sequential liquid–liquid extractions. The rapid extraction proposed by Lai *et al.* [8] does not give extracts clean enough to be analysed by high-performance liquid chromatography (HPLC).

This paper describes a new approach for the determination of bretylium in plasma, using solid-phase extraction and HPLC separation with UV detection. It also demonstrates the applicability of the method to pharmacokinetic studies, using the pig as an animal model.

EXPERIMENTAL

Chemicals and reagents

Bretylium tosylate was kindly provided by Burroughs Wellcome (Research Triangle Park, NC, USA). D-Tubocurarine (internal standard) was obtained from Sigma (St. Louis, MO, USA). Organic solvents and ammonium phosphate were of HPLC grade, and all other chemicals were of analytical grade (Fisher Scientific, Montreal, Canada).

Sample preparation

Bond-Elut CBA cation-exchange extraction cartridges (Analytichem International, Harbor City, CA, USA) were conditioned with a sequence of 3 ml methanol, 3 ml of water and 1 ml of 0.05 *M* borate buffered at pH 9.0. Human or porcine plasma (250 μl) and 0.2 μg of internal standard (200 μl) were added to the cartridges and allowed to be adsorbed on the column packing. The cartridges were washed successively with 3 ml of water, 1 ml of 0.05 *M* sodium dihydrogen phosphate buffer (pH 3.0) and 1 ml of water under a weak vacuum (< 20 kPa). Finally, a methanol rinse (2 × 0.5 ml) was performed to dehydrate the sorbent. The analytes were eluted into glass tubes with 2 × 0.5 ml of acidified methanol (833 μl HCl/100 ml methanol). The eluents were evaporated to dryness at 45°C under a gentle stream of nitrogen, and the residues were reconstituted in 200 μl of methanol–acetonitrile–water (30:15:55, v/v) adjusted to pH 3.4 with 1 *M* phosphoric acid. Volumes of 70 μl were injected into the chromatographic system.

Chromatography

The HPLC system consisted of a Constametric III pump (LDC Milton Roy, Riviera Beach, FL, USA) set to deliver 2.5 ml/min of mobile phase. A Model 7125 injector (Rheodyne, Cotati, CA, USA) was fitted with a 75-μl loop. A 10 cm × 4.9 mm I.D. reversed-phase column packed with 5-μm octyl Spherisorb (Hichrom, Reading, UK) was used for the separation of the analytes. The column was preceded by an SSI 0.45-μm in-line filter (State College, PA, USA). The absorbance of drugs was monitored with a variable-wavelength UV detector set at 272 nm (SM 4000, Milton Roy) connected to a C-R6A integrator (Shimadzu, Kyoto, Japan). The mobile phase was methanol and acetonitrile in an aqueous mixture of 0.01 *M* octanesulphonic acid (sodium salt) and 0.0015 *M* dibutylamine (30:15:55, v/v). The solution was adjusted to pH 3.4 with 1 *M* phosphoric acid, passed through a 0.45-μm filter (Millipore, Waters Assoc., Milford, MA, USA) and degassed. The chromatography was carried out at room temperature and the mobile phase was recirculated. The total HPLC run-time for bretylium analysis was less than 7 min.

Recovery

The recovery of bretylium from plasma following extraction was estimated in quadruplicate, at two different drug concentrations (1.25 and 20 μg/ml), by comparing the bretylium/internal standard peak-height ratio obtained after the extraction of spiked samples with the peak-height ratio obtained with the same amount of bretylium added directly to the tubes before evaporation. The recovery of the internal standard was assessed separately by comparing the peak-height response obtained after direct on-column injection of the same amount of drug.

Calibration curves

A pool of plasma containing 40 μg/ml bretylium tosylate was serially diluted with drug-free plasma to yield standards ranging from 0.625 to 40 μg/ml. Samples of 250 μl of each standard were extracted as outlined above and injected into the chromatograph. Calibration curves were obtained by least-squares regression of the bretylium/internal standard peak-height ratio *versus* bretylium concentration.

Precision and accuracy

Intra-assay variability was determined from replicate measurements ($n = 4$) of the 1.25 and 20 μg/ml drug concentrations used to assess recovery. The inter-assay variability was taken from calibration curves prepared on at least four different days.

Drug-free plasma was spiked with bretylium to yield ten different concentrations ranging from 0.9 to 40 μg/ml. Samples were blindly assayed and concentrations were derived from the calibration-curve. The accuracy was evaluated by comparing the estimated amount with the known drug concentration.

Limit of quantitation

The limit of quantitation was defined as the lowest plasma concentration of the standard curve. In order to estimate the minimum amount of drug detectable at a signal-to-noise ratio less than 3, a pool of plasma containing 1.25 μg/ml bretylium was also serially diluted with drug-free plasma to obtain concentrations of standards down to 0.156 μg/ml.

RESULTS AND DISCUSSION

Our method is, to our knowledge, the first one involving single-step extraction of bretylium from plasma applicable to HPLC analysis. Previous methods required the liquid–liquid removal of bretylium from plasma using ion-pairing reagents to salt out the drug selectively. In view of the cationic nature of bretylium, we investigated the possibility of employing potassium iodide as a pairing counter-ion prior to HPLC determination. A method used for the determination of vecuronium and requiring two sequential ion-pair extractions [9] was adapted for bretylium, and resulted in adequate sample clean-up but poor drug recovery. As an alternative to paired-ion extraction, we chose to evaluate open-column systems for cation-exchange extraction. CBA carboxylic acid cartridges provided the selectivity for the extraction of bretylium and D-tubocurarine, and proved to be optimal for obtaining extracts free of interfering substances at 272 nm. Similar results were obtained whether human or porcine plasma was used.

The clean-up efficiency of our method is evidenced in Fig. 1A, which represents a chromatogram obtained after the extraction of porcine blank plasma. As shown in Fig. 1C, the mobile

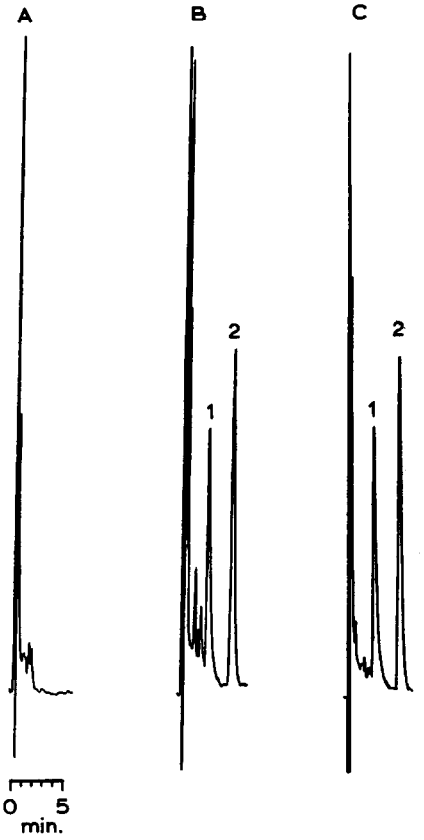

Fig. 1. HPLC of (A) porcine blank plasma, (B) porcine plasma sample collected 3 h after an intravenous bolus of 16.5 mg/kg bretylium tosylate and (C) human plasma spiked with 2.5 μg/ml bretylium. Peaks: 1 = bretylium; 2 = D-tubocurarine.

TABLE I

RECOVERY OF BRETYLIUM FROM PLASMA AND INTRA-ASSAY VARIABILITY

Amount added to 1 ml of plasma (μg)	Recovery (mean ± S.D., n = 4) (%)	Coefficient of variation (%)
1.25	75.0 ± 7.1	9.5
20.0	80.9 ± 2.9	3.6

phase in conjuction with a C_8 reversed-phase column resulted in excellent separation of bretylium (3 min) and D-tubocurarine (5 min) when an extract of human plasma spiked with 2.5 μg/ml bretylium tosylate was applied to the column. The presence of octanesulphonic acid in the mobile phase was essential for adequate on-column retention and separation of the drugs. Dibutylamine was added to decrease peak tailing.

The mean recoveries for a high and low concentration of bretylium (Table I) were 80.9 and 75.0%, respectively, indicating that the extraction efficiency was independent of the drug concentration. The recovery of D-tubocurarine from plasma was 72% (data not shown). The intra-assay precisions for samples containing 1.25 and 20 μg/ml were 9.5 and 3.6%, respectively (Table I).

The calibration curve for bretylium was linear over the concentration range investigated (Table II). Each concentration represents the average of four bretylium estimations. The regression equation for bretylium was $y = 0.309x - 0.039$ with a correlation coefficient (r^2) of 0.993. The inter-assay reproducibility was excellent and did not exceed 8% (Table II).

Blindly assayed spiked samples of bretylium at concentrations covered by the standard curves showed an accuracy of 101.0 ± 5.4% (mean ± S.D., n = 10).

Our analytical method was recently applied to the determination of plasma bretylium levels after the intravenous administration of 16.5 mg/kg bretylium tosylate to pigs (details of this study will be presented elsewhere). Briefly, blood samples were drawn in heparinized tubes for up to 4 h and kept frozen until assayed for bretylium. A typical chromatogram obtained from a 3-h plasma sample is shown in Fig. 1B. Fig. 2 illustrates the bretylium plasma concentration–time profile in the same pig.

The limit of quantitation of our assay is well beyond that required for pharmacokinetic studies in animals [7]. The lower limit of quantitation (0.625 μg/ml) could easily be increased by using larger sample volumes. Bretylium extracted from

TABLE II

BRETYLIUM CALIBRATION CURVE IN PLASMA AND INTER-ASSAY VARIABILITY

Concentration (μg/ml)	n	Peak-height ratio (mean ± S.D.)	Coefficient of variation (%)
0.625	5	0.188 ± 0.011	5.7
1.25	5	0.366 ± 0.010	2.7
2.5	4	0.786 ± 0.039	5.0
5	5	1.467 ± 0.068	4.6
10	4	2.926 ± 0.135	4.6
20	4	6.915 ± 0.377	5.5
40	4	12.335 ± 0.958	7.8

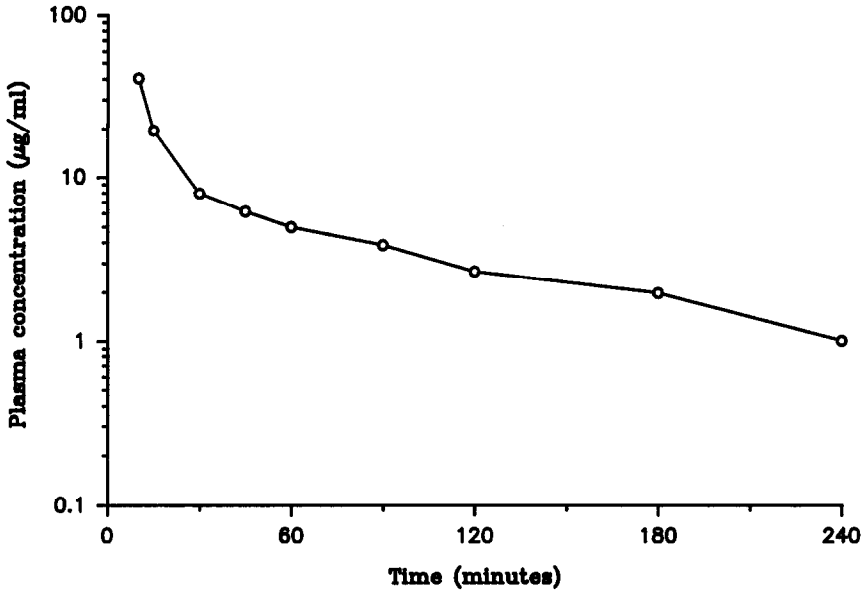

Fig. 2. Plasma concentration–time profile following intravenous administration of 16.5 mg/kg bretylium tosylate in one pig.

250 μl of plasma containing 0.156 μg/ml of the drug gave a signal-to-noise ratio of greater than 3, and was on the linear portion of the standard curve. Therefore, detection of low nanogram concentrations, which might be encountered in humans following therapeutic doses of bretylium [10], would be feasible with minor modifications to this assay.

CONCLUSION

This assay for bretylium is a major improvement over previous methods. Isolation of bretylium from plasma using solid-phase extraction cartridges is rapid and easy to execute. Drug detection relies on HPLC and UV absorbance and is therefore accessible to most laboratories. The procedure requires only small sample volumes to obtain the precision, selectivity and sensitivity required for pharmacokinetic studies in animals, and should prove useful to define further the pharmacokinetics of bretylium in humans. In addition, the use of cation-exchange cartridges may prove applicable to the selective extraction of other quaternary ammonium compounds.

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