High-Performance Liquid Chromatographic Analysis of Tocainide in Human Plasma

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Abstract: A method for the determination of tocainide, a new antiarrhythmic drug, by means of ion-pair High Performance Liquid Chromatography (HPLC), is described. The drug and its chemically related internal standard are extracted from the basified plasma sample with methylene chloride. The organic extract is evaporated to dryness, dissolved in the mobile phase and chromatographed on a μ-Bondapak C-18 reversed phase column under radial compression. The detection limit is 200 ng/ml. The main metabolites as well as some frequently used antiarrhythmic drugs do not interfere in the assay.

Tocainide, 2-amino-*N*-(2,6-dimethylphenyl)propamide (**1**), a primary amine with a structure analogous to lidocaine (**2**) (Fig. 1) is used for the treatment of chronic ventricular tachy-arrhythmias (1–4). Unlike lidocaine, antiarrhythmic activity is observed after giving the drug orally as well as intravenously. Because of the long plasma half-life of 11 to 14h (1, 2, 5), one can maintain convenient oral dosage regimens. The therapeutic plasma concentration is 3 to 9 μg tocainide (base)/ml (6–7). Since clinical effects correlate well with plasma concentrations (8–9), tocainide plasma levels may be monitored to achieve maximum therapeutic effect with minimum side-effects.

The quantitative analysis of tocainide in biological fluids is feasible with HPLC, using variable wavelength detectors (10–14), or fluorescence detection following derivatization with dansyl chloride (6) or fluorescamine (15), as well as with gas liquid chromatography, using flame ionization (16), electron capture (4, 20) or *N*-selective detection (21, 22). However, most published HPLC methods require time consuming extraction procedures (5 to 15 min), (6, 11–13), derivatization following repeated extractions (6, 15), or relatively large plasma volumes (500 μl or more) (11–14, 19). Gas liquid chromatographic methods also require derivatization following solvent extraction (1, 4, 16, 21), except for the assay described by Lesne et al. (22).

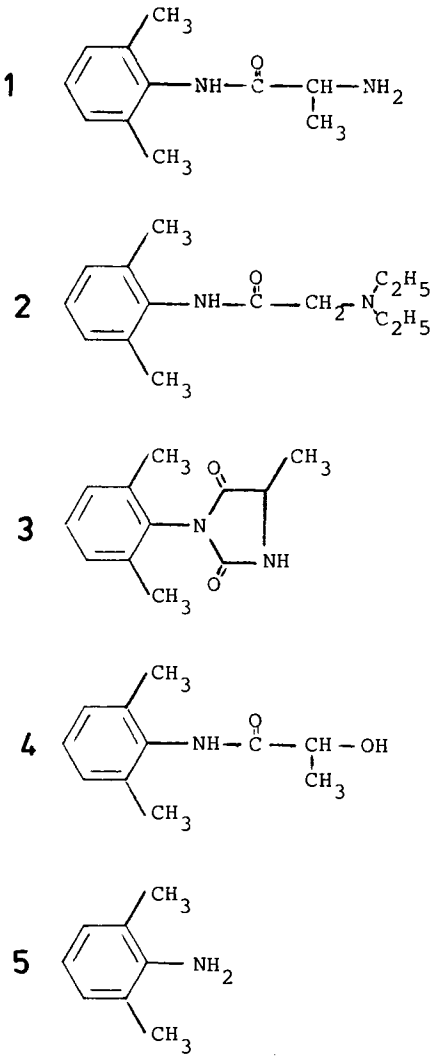

Fig. 1 Chemical structures of tocainide (**1**), lidocaine (**2**), tocainidehydantoine (**3**), lactoxylidide (**4**) and 2,6-dimethylaniline (**5**).

The new HPLC method described here requires no derivatization, demands comparatively small plasma samples and has a high degree of precision, linearity and reproducibility in the therapeutic range. Metabolites of tocainide can also be selectively determined by a modified HPLC method, under different extraction conditions. This method, however, is not described in this paper since no active circulating metabolites of tocainide have been identified.

Materials and Methods

Apparatus and Conditions of Analysis

The chromatograph consisted of an M-6000 A high pressure pump (Waters Associates), an M-7125 injector with a 60 μl loop (Rheodyne), a Radial Compression System (Z-module, Waters Associates), and a Lambda-Max 480 LC Spectrophotometer as detector, set at the wavelength of 264 nm (Waters Associates). The chromatograms were recorded on a Waters Data Module M-730 recorder/integrator. Separation was carried out on a μ-Bondapak C-18 (8MBC18 10 μm), reversed-phase column under radial compression with the mobile phase consisting of 6 mM sodium octanesulfonic acid in water, methanol, acetonitrile, acetic acid 96% (45:40:15:1) at a flow rate of 1.4 ml/min. All operations were done at ambient temperature.

Chemicals

The following drugs and metabolites were donated by the pharmaceutical companies indicated: tocainide HCl, lactoxylidide (H170/82), tocainidehydantoine (H170/84), *p*-methyl-tocainide HCl, glycinexylidide HCl, monoethylglycinexylidide, lidocaine HCl, metroprolol tartrate, quinidine bisulfate from Hässle (Sweden); disopyramide, desisopropyldisopyramide from Roussel (France); verapamil HCl, norverapamil HCl from Knoll (Germany); amiodarone HCl, desethylamiodarone oxalate from Labaz (Belgium); aprindine HCl, desethylaprindine HCl from Christiaens (Belgium); mexiletine HCl from Boehringer Ingelheim (Germany); propranolol HCl, *N*-desisopropylpropranolol HCl, 4-OH-propranolol from I.C.I. (Belgium); *N*-acetylprocainamide HCl from Janssen Chimica (Belgium); procainamide HCl from Federa (Belgium); sodiumoctanesulfonic acid 98%, and methanol 99.7% were purchased from Janssen Chimica (Belgium). Methanol was glass-distilled before use. HPLC quality acetonitrile was obtained from Farmitalia Carlo Erba (Italy). Water for

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HPLC was purified by a Millipore Milli-Q system. The remaining reagents were pro analysis quality.

Extraction

A plasma sample (100 to 300 μl) is introduced in a test-tube (14 × 200 mm). After adding 100 μl of internal standard solution (30 μg/ml *p*-methyl-tocainide base in acetonitrile), 100 μl NaOH 1N and 3 ml dichloromethane, the tube is shaken on a vortex mixer for 30 seconds. After centrifuging at 1700 g for 5 min, the water layer is discarded by aspiration. The organic layer is evaporated to dryness at room temperature (20°C) under a stream of air. The residue is dissolved in 75 μl mobile phase, and 60 μl is injected in the chromatograph.

Results and Discussion

Chromatograms

Ion-pair chromatography allows quantitation of tocainide as shown by Fig. 2.

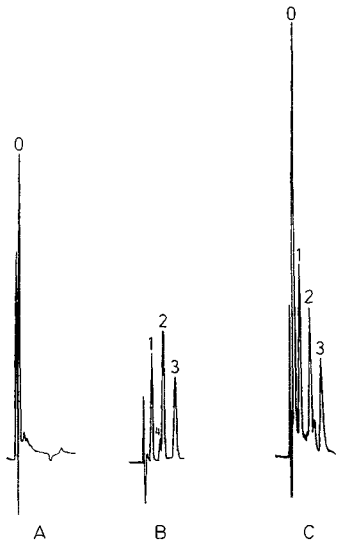

Fig. 2 Chromatograms
(A) Drug free plasma.
(B) Standard mixture in water:

1 tocainidehydantoine	5 μg/ml
1 lactoxylidide	5 μg/ml
2 tocainide	10 μg/ml
3 *p*-methyl-tocainide	30 μg/ml
4 2,6-dimethylaniline	500 ng/ml

(C) Plasma sample of a patient on chronic tocainide therapy.

0 plasma peaks	
1 tocainidehydantoine	R.T. 4.62 min
1 lactoxylidide	R.T. 4.40 min
2 tocainide	R.T. 6.33 min
3 *p*-methyl-tocainide	R.T. 8.36 min
4 2,6-dimethylaniline	R.T. 5.90 min

High circulating amounts of the metabolites tocainidehydantoine (**3**), lactoxylidide (**4**) and 2,6-dimethylaniline (**5**) (Fig. 1), not active as antiarhythmic agents, were found in some patients treated with tocainide. A modified HPLC analysis allows specific determination of all tocainide metabolites (data not shown).

Linearity Test and Reproducibility

The calibration curve was obtained by analyzing triplicate plasma samples containing a known concentration of tocainide base (Table I). The detector signal is linear up to a concentration of 20 μg tocainide per ml plasma. Linear regression analysis of the data (peak height ratio drug/internal standard) yields a straight line with an intercept of zero.

Table I. Calibration curve.

tocainide base[a] (μg/ml)	peak height ratio[b] ± S.D.[c]	equation y = ax + b
2.50	0.340 ± 0.018	slope = 7.3099
5.00	0.690 ± 0.011	y intercept
10.00	1.379 ± 0.050	= −0.0146
15.00	2.052 ± 0.051	correlation
20.00	2.736 ± 0.085	= 0.9998

[a] Internal standard (2 μg) was added to each sample.
[b] The peak height ratio is determined by peak height tocainide/peak height *p*-methyl-tocainide.
[c] The standard deviation was calculated from data in 3 experiments.

Table II. Intra-assay variability

Tocainide Base (μg/ml plasma)	Relative S.D.	Concentration Found (μg/ml Plasma) ± S.D. (n = 10)
1.50	12.1%	1.62 ± 0.20
2.50	4.0%	2.55 ± 0.10
6.00	1.7%	5.92 ± 0.10
8.50	1.5%	8.43 ± 0.13

Table III. Inter-assay variability, determined on six different days.

Tocainide Base (μg/ml Plasma)	Relative S.D.	Concentration Found (μg/ml Plasma) ± S.D. (n = 6)
1.50	8.60%	1.58 ± 0.14
5.00	1.50%	5.12 ± 0.08
7.50	1.90%	7.40 ± 0.14
10.00	1.60%	10.21 ± 0.16
15.00	2.40%	15.08 ± 0.36

Intra-assay variability was determined by analyzing 10 plasma samples containing a known concentration of tocainide (Table II). Inter-assay variability was determined by analyzing the samples on 6 different days (Table III).

The recovery of tocainide was 96.6% ± 3.3% (n=10) and 95.3% ± 2.4% (n=10) for *p*-methyl-tocainide.

External Quality Control

Fifty plasma samples of patients treated with tocainide HCl were analyzed by the method described in this paper. The results were compared with the results obtained with another HPLC method developed by the Department of Clinical Pharmacy of the Sittart Hospital (The Netherlands) (F. W. H. M. Merkus, personal communication). The correlation between the two methods was good, with r = 0.916.

Selectivity

There were no interfering peaks of endogenous origin. A chromatogram of a drug-free plasma extract is shown in Fig. 2A, while Fig. 2B depicts a chromatogram of standard samples of tocainide and its metabolites. Fig. 2C shows a chromatogram obtained from plasma of a patient treated with tocainide. The separation obtained by ion-pair chromatography allows selec-

Table IV.

Drug (Base)	Retention Time (min)
Lactoxylidide	4.40
Tocainidehydantoin	4.62
Procainamide	4.76
N-acetylprocainamide	5.50
Tocainide oxime	5.45
2,6-Dimethylaniline	5.90
Glycinexylidide	6.25
Tocainide	6.33
Desisopropyldisopyramide	6.51
Monoethylglycine	6.71
4-OH-Propranolol	6.76
Lidocaine	7.25
Metoprolol	7.40
Disopyramide	7.49
p-Methyl-tocainide	8.36
Mexiletine	9.65
Desisopropylpropranolol	9.69
Quinidine	9.95
Propranolol	12.27
Norverapamil	15.80
Verapamil	15.97
Desethylaprindine	27.75
Aprindine	31.59
Amiodarone	120.00
Desethylamiodarone	120.00

tive determination of tocainide in the presence of its metabolites.

Since antiarrhythmic drugs are frequently used in combination therapy, several common antiarrhythmic drugs and their metabolites were examined for possible interference (Table IV). All parent drugs were clearly separated, while two metabolites partially overlapped with tocainide.

Acknowledgements

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Prostaglandin D_2 Induced Potentiation of the Anticonvulsant Actions of Phenobarbitone and Phenytoin in Rats. Role of Serotonin

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Abstract: Prostaglandin D_2 (PGD_2) produced a dose-related potentiation of the anticonvulsant actions of sub-effective doses of phenobarbitone and phenytoin against maximal electroshock-induced seizures in rats. PGD_2-induced potentiation of phenobarbitone and phenytoin was significantly attenuated following pretreatment with centrally administered 5,6-dihydroxytryptamine, a selective neurotoxin for serotonergic neurones, *p*-chlorophenylalanine, a specific inhibitor of serotonin biosynthesis, and methysergide, a serotonin receptor antagonist, indicating that the potentiation was serotonin-mediated.

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Prostaglandins (PGs) are now known to exert a variety of physiological functions in the mammalian central nervous system (CNS), modulation of central neuronal activity being one of them (1, 2). Catecholamines and serotonin stimulate the biosynthesis and release of PGs (2, 3), and the latter are known to influence central catecholaminergic and serotonergic activities (4–6). Till recently PGs of the E and F series had attracted maximal scientific interest in relation to mammalian brain functions (1). However, it is now evident that there is considerable species variation in the distribution of central PGs and that PGD_2 is by far the most dominant PG in the rat and mouse brain, the levels of PGE_2 and $PGF_{2\alpha}$ being substantially lower (7). Recent studies indicate that PGD_2 shares some of the behavioral effects shown by PGs of the E series (8). PGD_2, like PGE_1, has a sedative effect in rodents and potentiates pentobarbital hypnosis (9–11). PGE_1 has been shown to potentiate the anticonvulsant action of phenobarbitone in rats by a serotonin-mediated mechanism (12). We have recently shown that, like PGE_1 (5), PGD_2 increases the serotonin concen-