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Note

Fluorometric liquid chromatographic assay of the antiarrhythmic agent flecainide in blood plasma

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Flecainide [R818, the acetate salt of 2,5-di-(2,2,2-trifluoro-ethoxy)-N-(2-piperidylmethyl)benzamide] is a novel antiarrhythmic agent. It suppresses induced arrhythmias in dogs and pigs [1—3]. The antiarrhythmic properties of the drug are currently being tested in patients [4], but a good assay has not been reported. Gas chromatography has been used [2, 4, 5]. However, no details are given in the literature. We combined the resolving power of high-performance liquid chromatography with the sensitivity of fluorescence detection to assay flecainide in deproteinized blood plasma. This paper describes a rapid, sensitive and accurate method which is relatively specific, and is linear over a very wide concentration range.

EXPERIMENTAL

Reagents

Flecainide acetate was obtained from Riker Laboratories (Loughborough, Great Britain), and H_3PO_4, KOH, K_2CO_3, $HClO_4$ and methanol (LichroSolv) were obtained from Merck (Darmstadt, G.F.R.). $NH_4H_2PO_4$ was supplied by Baker Chemical Co. (Phillipsburg, NJ, U.S.A.). Water was purified with the Milli-RO/Milli-Q System (Millipore, Bedford, MA, U.S.A.). The elution buffer consisted of a mixture of 600 ml of 50 m*M* $NH_4H_2PO_4$ (adjusted to pH 3.0 with H_3PO_4) and 400 ml of methanol; before use, the buffer was filtered through a 0.45-μm filter (Millipore).

Apparatus

A Model 5010 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a Valco injector (Valco Instruments Co., Houston, TX, U.S.A.) was

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used. Fluorescence was measured with an Aminco SPF-500 ratio detector (American Instrument Cy., Silver Spring, MD, U.S.A.) with an Aminco J4-9618 Microcell Condensing System Accessory. Some studies were conducted with a Vari-Chrom UV detector (Varian). Fluorescence emission or UV absorbance was monitored with a Servigor S RE543 chart recorder (Goerz Electro AG, Vienna, Austria). A C_{18} μBondapak column (particle size 10 μm; Waters Assoc., Milford, MA, U.S.A.) was cut into half to shorten the analysis time. Final dimensions of the column were 15 × 0.4 cm.

Procedure

Heparinized blood plasma was stored at −20°C until analyzed. A 1-ml aliquot was deproteinized with 1.0 ml of 0.8 *M* $HClO_4$ at 0°C. The mixture was shaken for 1 min and centrifuged for 4 min at 5500 *g* using an Eppendorf 3200 table centrifuge (Eppendorf, Hamburg, G.F.R.). An aliquot of the supernatant fluid (1.5 ml) was adjusted to pH 5—7 with 140 μl of a 6 *M* KOH—1 *M* K_2CO_3 solution. After centrifugation at 5500 *g* for 4 min, 200 μl of the supernatant fluid were applied to the column.

Chromatographic conditions

The flow-rate was 2.0 ml/min and the column was kept at 30°C. Under these conditions the retention time of flecainide is about 6 min. The fluorescence detector was set at an excitation wavelength of 300 nm and an emission wavelength of 370 nm. The excitation and emission slits on the monochromators were set at 10 and 15 nm bandwidths, respectively. UV absorption was measured at 280 nm. Calibration with standards was done every five determinations.

RESULTS AND DISCUSSION

Chromatograms

Fig. 1 shows chromatograms obtained with a plasma blank and plasma spiked with 200 ng/ml flecainide.

Linearity

Standards added to buffer or plasma gave a linear response up to 10 μg/ml when 200-μl samples were injected (Fig. 2). The sensitivity of the technique permits the detection of 50 ng/ml plasma flecainide, the detection limit being defined as a signal three times the height of the noise level.

Recovery

Plasma was deproteinized with $HClO_4$ and subsequently neutralized as described in the Experimental section. This procedure did not affect flecainide in water or buffer (recovery > 98%). We conclude from the calibration curves of flecainide in buffer and plasma (Fig. 2) that 35% of the drug was precipitated with plasma protein due to the $HClO_4$, but that this was independent of the flecainide concentration. Thus the recovery over the whole concentration range studied was 65.4 ± 3.2% (mean ± S.D., n = 17). Deproteinization with HCl, trichloroacetic acid, methanol, ethanol or acetone did not improve this recovery.

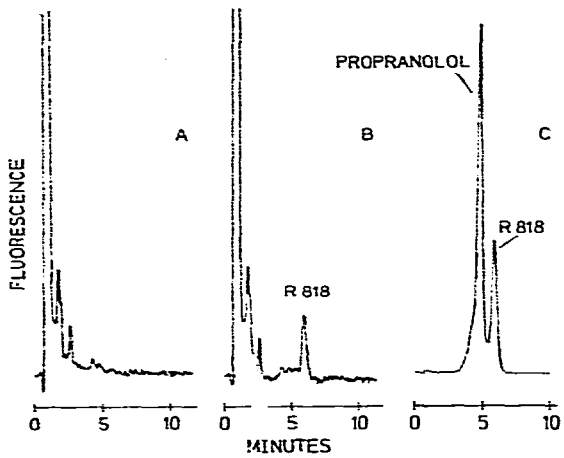

Fig. 1. Chromatograms obtained by high-performance liquid chromatography of (A) plasma blank, (B) plasma spiked with 200 ng/ml flecainide (R818), and (C) propranolol and flecainide in elution buffer (500 ng/ml). For deproteinization of plasma, see Experimental.

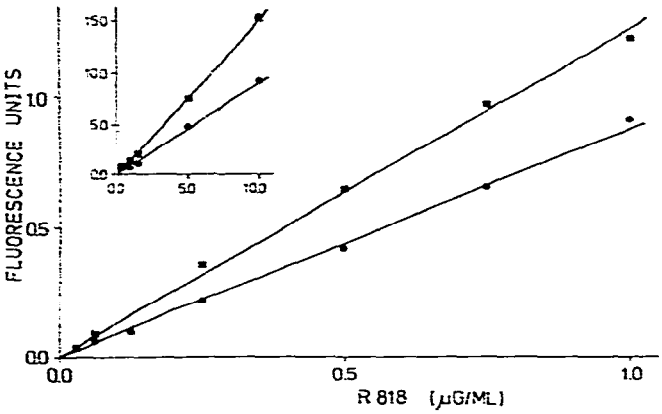

Fig. 2. Calibration curves of flecainide up to 10 µg/ml added to elution buffer (▪) or plasma (•). After the addition of the drug, plasma was deproteinized as described under Experimental.

Interference

Table I lists drugs that have been tested for potential interference with the procedure. All these drugs, including β-blocking agents, heart glycosides, vasodilators and antithrombolytics, could be detected by UV absorption, but only a number of them were fluorescent under the assay conditions. Propranolol showed considerable fluorescence; however, the separation from flecainide is adequate (Fig. 1), as is the case for the other drugs (Table I).

Precision

Inter-assay precision expressed as relative standard deviation (coefficient of variation) was 2.2, 1.2 and 1.2% for the 100, 500 and 1000 ng/ml aqueous standards, respectively (n = 5). For plasma flecainide determinations (concentration range 400—1500 ng/ml) both intra- and inter-assay standard deviation was found to be about 40 ng/ml.

TABLE I

DRUGS TESTED FOR THEIR POTENTIAL INTERFERENCE WITH THE FLECAINIDE ASSAY

Drug	UV detection		Fluorescence detection	
	Concentration tested (μg/ml)	Relative retention time(s)	Concentration tested (μg/ml)	Relative retention time(s)
Flecainide acetate	10	1.00	1	1.00
Acetylsalicylic acid	3600	0.43	50	0.40
Caffeine	1000	0.26	1000	NPD**
Diazepam	10	0.31; 0.49	600	NPD
Digitoxin	20	0.29; 0.40	200	NPD
Digoxin	2.5	0.29; 0.40	250	NPD
Dipyridamole	75	0.21; 0.23	75	NPD
Disopyramide phosphate	10	5.00	1000	NPD
Fenprocoumon	1500	0.09; 0.14	300	0.20
Heparin (sodium salt)	500 IU	0.46	100 IU	1.83
Nifedipine	100	2.57*	25	NPD
Nitroglycerin	5	0.17	5000	0.19
Practolol	500	0.16; 0.37	500	NPD
Procainamide chloride	10,000	0.20	1000	0.25
Propranolol chloride	10	0.86	0.5	0.83
Quinidine sulfate	150	0.30*	1	0.47*
Sulfinpyrazone	10,000	0.11; 2.40	20,000	NPD
Thiazide chloride	10,000	0.14	5000	NPD

*Relative retention time of main peak; other peaks observed with relative retention times < 0.90 and > 1.10.
**NPD = no peak detected.

Plasma concentrations

In patients treated with flecainide we found plasma concentrations of 540 ± 180 ng/ml (mean ± S.D., n = 60). The therapeutic concentrations in human plasma probably range between 180 and 900 ng/ml (cf. refs. 4—6), which means that subtherapeutic concentrations and overdoses can also be determined with the method described.

CONCLUSION

This assay based on high-performance liquid chromatography with fluorescence detection is rapid, sensitive and relatively specific. It seems useful in estimating plasma flecainide levels for clinical management.

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