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Note

Determination of alinidine in human plasma by high-performance liquid chromatography

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The N-allyl derivative of clonidine (alinidine, ST 567) produces substantial bradycardia in several species [1, 2] and in man [3, 4]. This bradycardic effect is not mediated by alpha- and beta-adrenoreceptors or by muscarinic receptors [3, 5]. Unlike beta-adrenoreceptor blocking agents and calcium antagonists this bradycardia is not accompanied by a depressant effect on the AV-nodal conduction [3]. A substance of such a pharmacological profile may be of clinical interest. Preliminary studies in patients with coronary heart disease indicate that the use of alinidine may represent a new kind of antianginal therapy [4].

We describe here an assay for alinidine using high-performance liquid chromatography (HPLC). The assay permits studies of the pharmacokinetics of this agent in man.

EXPERIMENTAL

Reagents and materials

All chemicals and solvents were analytical grade quality (E. Merck, Darmstadt, G.F.R.) and were used without further purification. Alinidine · HBr, [^{14}C] alinidine · HBr (specific activity 36 μCi/mg), and 2-[N-(thienyl-2-methyl)-N-(2,6-dibromophenyl)-amino]-imidazoline · HCl (STH 2199-Cl) which is shown in Fig. 1 were generously supplied by C.H. Boehringer Sohn (Ingelheim, G.F.R.). Aqueous solutions were prepared in double distilled water. All glassware used during the sample preparation was silanized with dichloromethylsilane, rinsed with toluene—methanol and several times with methanol and

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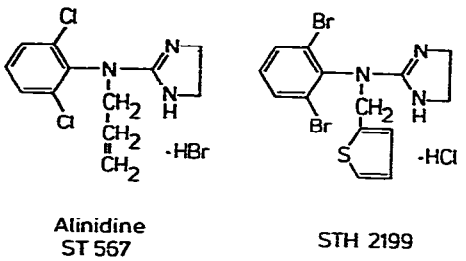

Alinidine
ST 567

STH 2199

Fig. 1. Chemical structures of alinidine • HBr and STH 2199-Cl.

double distilled water. The PTFE linings of the screw-capped culture tubes were ultrasonicated in methanol and double distilled water and rinsed extensively.

Sample preparation

The plasma samples (1 ml) were transferred to PTFE-lined screw-capped culture tubes containing 5 μl of internal standard STH 2199-Cl (about 300 ng). Fifty microlitres of 5 *N* sodium hydroxide and 5 ml of diethyl ether were added. The samples were extracted for 25 min on a rotary mixer at 25 rpm and centrifuged at 1000 *g* for 10 min. The ether phase was separated from the aqueous phase and extracted twice with 2 ml of 0.01 *N* hydrochloric acid under the same conditions as before. The combined aqueous phases were alkalinized with 50 μl of 5 *N* sodium hydroxide and extracted with chloroform under the same conditions. The aqueous layer was aspirated. The chloroform phase was transferred to a conical glass tube and evaporated under a gentle nitrogen stream. The walls of each tube were rinsed with 200 μl chloroform and evaporated again. The procedure was repeated with 100 μl chloroform. The residues were dissolved in 25 μl mobile phase, agitated on a Vortex mixer for 60 sec and centrifuged briefly. A 20-μl aliquot of the extract was injected into the chromatograph.

Chromatography

A Waters Model 6000A high-pressure solvent delivery system was used equipped with a Model U6K injector and a Model 450 variable-wavelength detector fitted with a μBondapak C_{18} reversed-phase column (30 cm × 3.9 mm I.D., particle size 10 μm) (Waters Assoc., Milford, MA, U.S.A.). The absorbance was measured at 210 nm. The mobile phase was 50 m*M* sodium phosphate in methanol—water (50:50) adjusted to pH 5.0 with hydrochloric acid and filtered through an 0.45-μm filter before use. The detector output was recorded at 10 mV at a chart speed of 5 mm/min. All chromatography was performed at ambient temperature.

Calibration

Calibration curves were constructed by adding known amounts of alinidine • HBr (10, 20, 30, 50, 75, 100, 125 and 150 ng/ml) to pooled human plasma. The peak height ratios of alinidine • HBr to the internal standard were plotted against the concentration of alinidine • HBr. The least squares regression line was fitted through the data points. The alinidine concentrations of the un-

known samples were determined by using the regression equation of the calibration curve which was assayed concurrently with the unknown samples. Plasma samples containing concentrations higher than 150 ng/ml were adequately diluted with blank plasma.

Application of the method

A healthy volunteer received 80 mg alinidine · HBr intravenously and as a tablet. The crossover studies were carried out four weeks apart. The subject had taken no drugs for at least two days before the study, refrained from smoking on the days of the study, and had fasted overnight. Blood samples were taken frequently after dosing from an antecubital vein using silanized plastic syringes and heparin (5 U/ml blood) as anticoagulant. The plasma was separated by centrifugation and stored at −20°C until assayed.

RESULTS AND DISCUSSION

The resolution of the chromatographic system was checked daily by injection of 20 μl of a mixture containing alinidine · HBr and the internal standard. Alinidine and STH 2199 showed retention times of 4.3 and 5.9 min, respectively (Fig. 2). Using the extraction method described above endogenous plasma components did not interfere with alinidine or the internal standard (Fig. 2). The detection limit for alinidine (using a signal-to-noise ratio of 4) was about 5 ng/ml.

The linearity of the detector response was assessed by injecting 20 μl of alinidine solutions with concentrations ranging from 10—500 ng/ml. The absolute peak height of alinidine was plotted against the concentration. The relationship was linear and passed through the origin. Calibration curves were obtained by plotting the peak height ratios of alinidine and STH 2199 versus the alinidine concentrations. In the investigated concentration range (10—150 ng/ml) the regression line was linear (r = 0.995) with an intercept on the y-axis close to the origin (0.0064).

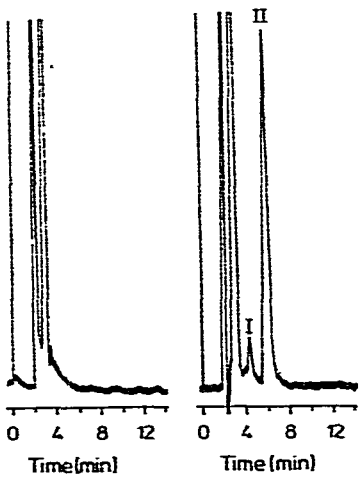

Fig. 2. Chromatograms of control plasma (left panel) and plasma containing 10 ng/ml of alinidine · HBr (I) and 295 ng/ml of STH 2199-Cl (II) (right panel).

The precision of the method was evaluated in a blind study in the concentration range of 10—500 ng alinidine per ml. The experimentally determined concentrations agreed sufficiently with the theoretical concentrations (Table I). The day-to-day variation of the assay stayed in an acceptable range. The slope of the standard curve showed a coefficient of variation of 7.4% ($n = 10$) within a time period of six weeks.

The recovery of the extraction procedure as described above was estimated by using [^{14}C] alinidine. The recovery was 77.2 ± 5.9% ($n = 24$) in the concentration range 10—400 ng/ml.

TABLE I

PRECISION OF THE ASSAY

Theoretical alinidine · HBr concentrations (ng/ml)	Experimentally determined alinidine · HBr concentrations* (ng/ml)	n	Accuracy** (%)
6.06	6.96 ± 2.92	6	14.8
94.29	89.17 ± 4.16	6	5.4
258.80	254.81 ± 22.94	12	1.5
553.13	530.41 ± 12.45	7	4.1

*Mean ± S.D.

**Calculated according to ref. 6.

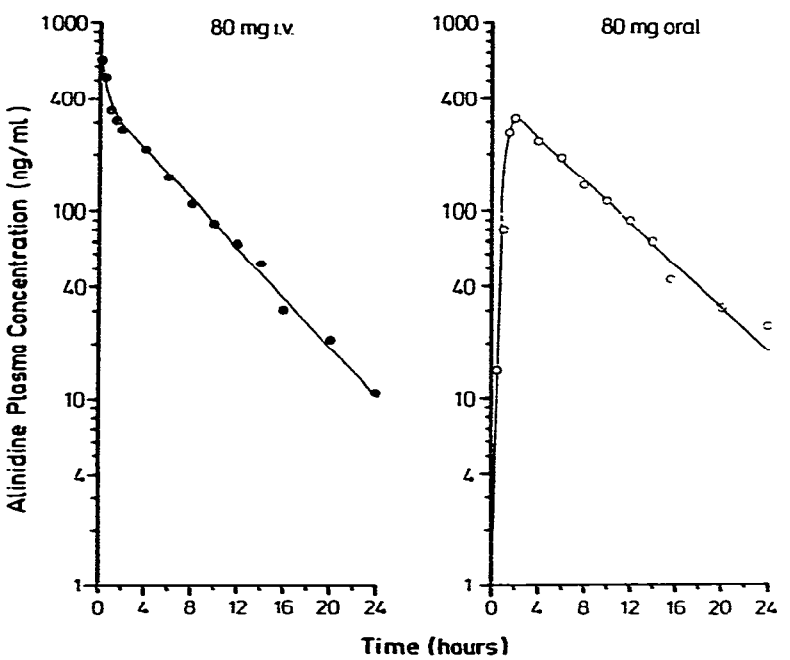

Fig. 3. Semi-logarithmic plot of plasma concentrations after an intravenous (•) and an oral (○) dose of 80 mg alinidine.

The application of the assay is demonstrated for one subject who received 80 mg alinidine intravenously and orally. Fig. 3 shows the plasma concentration—time curves for the intravenous and the oral administration of alinidine. In this particular subject the biological half-life of alinidine was 4.6 h for the intravenous dose and 5.8 h for the oral dose. The plasma clearance was 484 ml/min and the volume of distribution was 3.87 l/kg. A nearly complete bioavailability (94%) was calculated. Studies in man using radiolabeled alinidine gave a bioavailability of 100% and a mean biological half-life of 3.5 h [7]. Preliminary results from subjects who received 40 mg of alinidine gave pharmacokinetic parameters in a similar range. The data shown in this paper demonstrate that the HPLC method can be applied to pharmacokinetic studies of this new compound.

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