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Monitoring of ajmaline in plasma with high-performance liquid chromatography

Claus Köppel*

Reanimationszentrum, Universitätsklinikum Rudolf Virchow, Standort Charlottenburg, Freie Universität Berlin, Spandauer Damm 130, D-1000 Berlin 19 (Germany)

Angelika Wagemann

Landesuntersuchungsinstitut für Lebensmittel, Arzneimittel und Tierseuchen Berlin, Invalidenstrasse 60, D-1000 Berlin 21 (Germany)

Günter Roland Hansen and Christian Müller

Institut für Klinische Chemie und Biochemie, Universitätsklinikum Rudolf Virchow, Standort Charlottenburg, Freie Universität Berlin, Spandauer Damm 130, D-1000 Berlin 19 (Germany)

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ABSTRACT

A rapid, reliable and sensitive assay for routine determination of ajmaline in plasma by high-performance liquid chromatography with fluorimetric detection is presented. A low limit of detection in plasma (less than 1 ng/ml ajmaline) could be achieved by the extraction of plasma samples and the use of fluorimetric detection. Deproteinization of the plasma sample instead of extraction, or the use of an ultraviolet detector, yielded a higher limit of detection (less than 50 ng/ml). Two different eluents were studied. Eluent 1 allowed clear separation of ajmaline from isoajmaline and sandwicine, but did not separate isoajmaline from sandwicine. With eluent 2, separation of isoajmaline and sandwicine was achieved, but separation of ajmaline from sandwicine was less optimal than with eluent 1. Therefore, eluent 1 was used for further clinical studies. No interference was observed from therapeutic doses of other commonly co-administered drugs, such as acetylsalicylic acid, digoxin, digitoxin, ranitidine, dopamine, dobutamine, furosemide, captopril or glycerol trinitrate. In addition, the chemical stability of ajmaline and a possible rearrangement of ajmaline to its stereoisomers isoajmaline and sandwicine was studied *in vivo* and *in vitro*. Ajmaline proved to be unusually stable under both *in vivo* and *in vitro* conditions.

INTRODUCTION

Ajmaline is a potent intravenous antiarrhythmic drug with class IC as well as IA properties, according to Vaughan-Williams. It is widely used in Germany and many European countries as well as Japan for treatment of tachycardia in preexcitation syndromes and ventricular arrhythmia with acute onset not responding to lidocaine [1–6]. Despite its use since the early 1960s, few data are available on its clinical pharmacokinetics. This is in part due to the lack of a reliable and specific assay for ajmaline in plasma on a routine basis. We have therefore developed an ajmaline plasma assay, which has been used for establishing the pharmacokinetics of ajmaline in intensive care patients [7]. Since it is not known if ajmaline rearranges under extraction conditions to its naturally occurring isomers isoajmaline and sandwicine, the stability of ajmaline was also studied (Fig. 1). It should be noted that isoajmaline is thermodynamically more stable than ajmaline.

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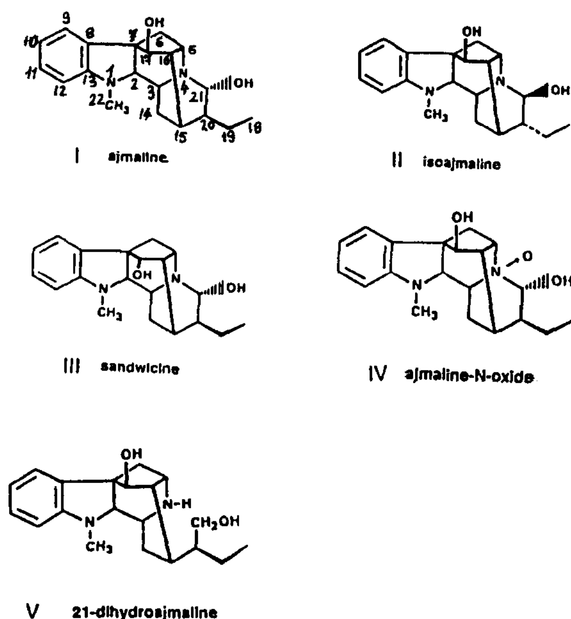

Fig. 1. Structures of ajmaline (I), isoajmaline (II), sandwicine (III), ajmaline N-oxide (IV) and dihydroajmaline (V).

For assaying ajmaline in biological fluids, methods involving thin-layer chromatography (TLC), paper chromatography, UV photometry and fluorimetry have been described, but they all lack sufficient selectivity and/or sensitivity [8–15]. Furthermore, it remains unclear whether this type of assay allows a distinction between ajmaline, its isomers and its metabolites. For detection of ajmaline in plant extracts, a radioimmunoassay has been described [16]. Additionally, polarography has been used [17]. A high-performance liquid chromatography (HPLC) assay has been used in a study [18] investigating the interaction of quinidine with ajmaline in plasma after oral administration: co-administration of quinidine enhances the poor oral bioavailability of ajmaline by a factor of *ca.* 18.

Only *ca.* 5% of ajmaline is excreted unchanged in the urine [8,9]. The main metabolic pathways are mono- and dihydroxylation with subsequent O-methylation, reduction of the C-21 hydroxy group, N-demethylation, oxidation of the hydroxyl groups and a combination of these pathways [19,20]. Ajmaline N-oxide is probably the only active metabolite. The poor oral bioavailability of ajmaline is due to an extensive first-pass metabolism. Ajmaline metabolism cosegregates with oxidative polymorphic sparteine/debrisoquine/dextromethorphan metabolism [20]. Polymorphic metabolism of this type has also been demonstrated for the ajmaline analogue prajmaline [21].

EXPERIMENTAL

Reagents and chemicals

Pure samples of ajmaline (I), isoajmaline (II), sandwicine (III), ajmaline N-oxide (IV) and dihydroajmaline (V) were kindly provided by Kali-chemie (Hannover, Germany). The purity of all reference compounds was checked by gas chromatography–mass spectrometry and ^{1}H NMR spectroscopy [20]. Additionally, the reference compounds were analysed by TLC using silica-gel plates (Merck, Darmstadt, Germany). The eluent was a mixture of 80% toluene, 20% methanol and 1% concentrated aqueous ammonia. Detection was made with iodoplatinate reagent (6 g of potassium iodide, 3 ml of 10% hexachloroplatinic acid and 4 ml of 38% hydrochloric acid in 200 ml of water).

The purity of the reference compounds was not less than 97%. All reagents, analytical grade or better, were purchased from commercial sources and used without further purification.

Sample preparation

For analysis, 0.5 ml of 0.1 *M* glycine buffer (pH 10) was added to 0.2–0.5 ml of plasma and extracted twice with 5 ml of diethyl ether (Nanograde, Mallinckrodt, Hennef, Germany). The organic solvent was carefully dried with sodium sulphate and then removed with a dry stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase. A 20-μl aliquot was used for a HPLC run.

As an alternative to extraction, 0.2 ml of plasma was vortex-mixed for 3 min with 0.3 ml of acetonitrile, allowed to stand for 10 min, vortex-mixed again and then centrifuged for 5 min at 3000 *g*. The clear supernatant was used for HPLC analysis.

High-performance liquid chromatography

A high-performance liquid chromatograph consisting of a Rheodyne 7125 (20-μl) loop, a 410 pump, a 235 diode array UV detector, an NS4 fluorimeter and an LCI-100 integrating recorder from Perkin Elmer (Ueberlingen, Germany) was used. The column (12 cm × 0.4 cm I.D., operated at room temperature), which was combined with a precolumn (2 cm × 0.4 cm I.D.), was packed with 5-μm Nucleosil C_{18} (Macherey & Nagel, Düren, Germany). The mobile phase was acetonitrile (Chrom AR, Mallinckrodt)–0.07% orthophosphoric acid (22:78, v/v) (eluent 1). The flow-rate was 1.5 ml/min. Alternatively, aqueous 0.2 *M* trifluoroacetic acid–acetonitrile (80:20, v/v) (eluent 2) was used, at a flow-rate of 1.1 ml/min.

The UV detector was operated at a wavelength of 245 nm with peak purity control by UV spectrum. Quantitation with the fluorimetric detector was done at an excitation wavelength of 296 nm and emission wavelength of 358 nm.

Calibration and quantitation

Standard stock solutions were prepared in methanol and diluted with the mobile phase. For determination of the calibration curve, pooled blank plasma was spiked with increasing concentrations of ajmaline (2, 5, 10, 50, 100, 250, 500, 750 and 1000 ng/ml). The recovery was determined by comparison of the peak area of the ajmaline standard stock solution with that of blank plasma spiked with ajmaline. The day-to-day precision was determined on days 1, 3, 5, and 8 by repeated triple analysis of plasma samples from patients. The plasma samples were kept at −20°C before analysis. The limit of detection was determined by spiking pooled blank plasma with decreasing concentrations of ajmaline. The recovery was estimated by comparison of values from an aqueous solution of ajmaline (50 ng/ml) and from a blank plasma sample with ajmaline (50 ng/ml).

Chemical stability of ajmaline

The *in vitro* stability of ajmaline was studied after refluxing of 5 mg of ajmaline hydrochloride in 10 ml of 25% hydrochloric acid for 30 min. In a further experiment, 5 mg of ajmaline hydrochloride were dissolved in a mixture of 10 ml of 0.1 *M* sodium hydroxide and 2 ml of methanol and stirred for 24 h at room temperature. Both reaction mixtures were extracted as described above.

Ajmaline, isoajmaline and sandwicine in the *in vitro* experiments were assayed by HPLC and TLC as described above.

Clinical studies

Six patients with acute myocardial infarction and ventricular arrhythmia of acute onset refractory to 100 mg of intravenous (i.v.) lidocaine, received an infusion of 20 mg/h ajmaline. After 24 h, blood from a central venous line was collected into heparinized tubes and centrifuged, and the plasma was stored at –20°C until analysis.

RESULTS

Chemical stability of ajmaline

Ajmaline was stable under acidic and basic conditions. No conversion of ajmaline into its thermodynamically more stable isomer isoajmaline or into sandwicine was observed. (The limit of detection for the identification of impurities was 4%.) The R_F values of ajmaline, isoajmaline, sandwicine, ajmaline N-oxide and dihydroajmaline under the TLC conditions were 0.56, 0.38, 0.46, 0.28 and 0.53, respectively.

HPLC assay

Ajmaline was clearly separated from isoajmaline and sandwicine by eluent 1 (Fig. 2). However, it was not possible to separate isoajmaline and sandwicine with this eluent. The retention times were 5.88 min for ajmaline (I), 5.10 min for isoajmaline (II) and sandwicine (III), 4.35 min for ajmaline N-oxide (IV) and 7.05 min for dihydroajmaline (V). Separation of sandwicine and isoajmaline but incomplete separation from ajmaline could be achieved with eluent 2 (Fig. 3). The retention times were 10.3 min for I, 9.4 min for II, 9.72 min for III, 11.81 min for IV and 8.7 min for V. Since optimal separation of ajmaline

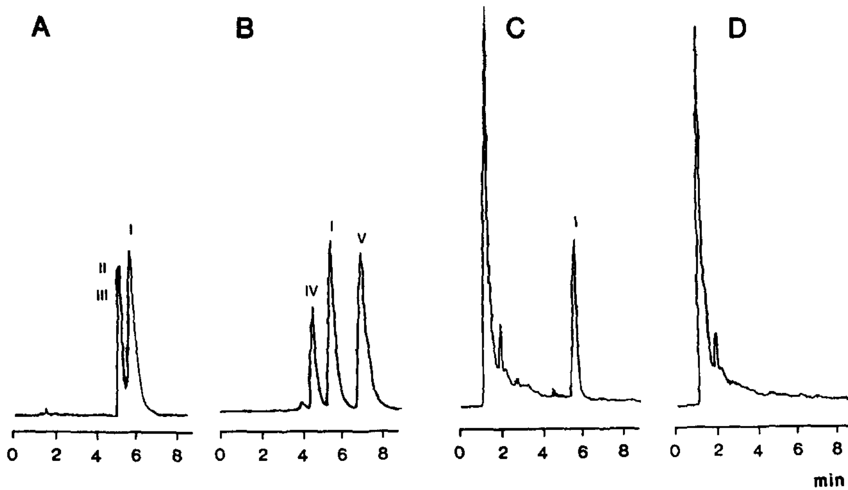

Fig. 2. HPLC of a standard solution of 10 ng/ml ajmaline (I), 5 ng/ml isoajmaline (II) and 5 ng/ml sandwicine (III) (A), a standard solution of 10 ng/ml ajmaline (I), 20 ng/ml ajmaline N-oxide (IV) and 20 ng/ml 21-dihydroajmaline (V) (B), a plasma extract from a patient (13 ng/ml ajmaline) (C) and a blank plasma extract (D) by eluent 1.

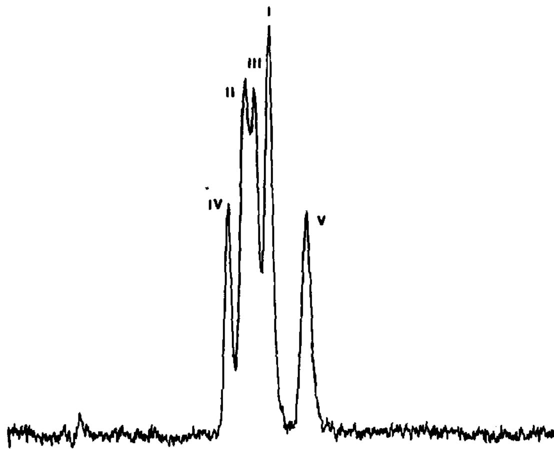

Fig. 3. Separation of a standard solution of 40 ng/ml ajmaline (I), 30 ng/ml isoajmaline (II), 10 ng/ml sandwicine (III), 50 ng/ml ajmaline N-oxide (IV) and 20 ng/ml dihydroajmaline (V) by eluent 2.

from its stereoisomers was considered to be more important, eluent 1 was used exclusively for further clinical studies.

Using the fluorimetric detector, the limit of detection for ajmaline (eluent 1) was 1 and 50 ng/ml with and without extraction, respectively. A relative standard deviation of 4% at 100 ng/ml ajmaline (ten determinations) and a day-to-day precision of ±6% were determined. Reanalysis of plasma samples on days 3, 5 and 8 gave no evidence for the decay of the ajmaline plasma concentration, if plasma samples had been stored at −20°C prior to analysis. The calibration curve was linear (r = 0.99) in an ajmaline concentration range of 2–1000 ng/ml. The mean recovery (ten determinations) at an ajmaline plasma level of 200 ng/ml was 99%, and 92% with deproteini-

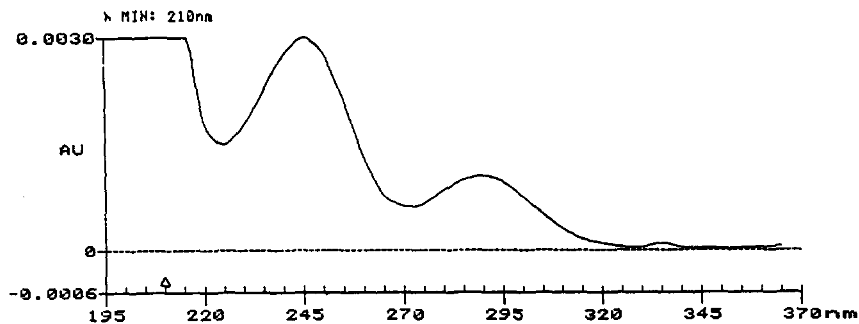

Fig. 4. UV spectrum of ajmaline (diode array detector).

zation or extraction, respectively. The estimated recovery was 97% for deproteinization and 94% for extraction. Drugs currently administered to intensive care patients, such as 100 mg of acetylsalicylic acid (orally, p.o.), 0.1 mg digoxin (p.o.), 0.4 mg digitoxin (p.o.), 10 mg/h dopamine (i.v.), 40 mg/h dobutamine (i.v.), 3 × 50 mg ranitidine per day (p.o.), 80 mg/h lidocaine (i.v.), 40 mg/h furosemide (i.v.), 3 × 10 mg diazepam per day (p.o.), 3 × 2 mg flunitrazepam per day (i.v.), 3 × 6.25 mg captopril per day (p.o.) and 3 mg/h glycerol trinitrate (i.v.) did not interfere with the HPLC assay. The limit of detection was considerably higher with UV detection or after deproteinization of the plasma sample and fluorimetric detection (less than 50 ng/ml ajmaline after extraction). A UV spectrum of ajmaline is depicted in Fig. 4.

Patients

In the six patients on a continuous infusion of 20 mg/h ajmaline, plasma levels after 24 h ranged from 0.25 to 0.95 μg/ml. In the plasma of these patients, isoajmaline, sandwicine, ajmaline N-oxide and dihydroajmaline were not detectable.

DISCUSSION

Ajmaline was unusually stable to isomerization under *in vivo* and *in vitro* conditions. The HPLC assay with eluent 1 allowed clear separation of ajmaline from its two isomers isoajmaline and sandwicine as well as its metabolites ajmaline N-oxide and dihydroajmaline. Separation of isoajmaline and sandwicine could be achieved with eluent 2 at the price of a poorer separation of ajmaline from sandwicine.

CONCLUSION

HPLC with fluorimetric detection is highly specific and allowed the sensitive detection of ajmaline for pharmacokinetic studies. An extraction procedure is not necessary if ajmaline plasma concentrations are expected to exceed 50 ng/ml, which facilitates routine monitoring of ajmaline plasma levels in patients. No interference with the detection of ajmaline was found from commonly co-administered drugs.

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