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Short Communication

Plasma level monitoring of D,L-verapamil and three of its metabolites by reversed-phase high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic assay was developed for determination of verapamil, norverapamil (M1) and its N-dealkylated metabolites (M2 and M3) in plasma. Plasma samples were vortex-mixed, deproteinized and centrifuged. The analysis was performed on a C_{18} reversed-phase column with fluorimetric detection. Since the polarity of verapamil and norverapamil differs considerably from that of M2 and M3, two different eluents were used for rapid high-performance liquid chromatographic separation. The eluent for the separation of verapamil and norverapamil was acetonitrile–0.07% orthophosphoric acid (33:67, v/v), and for M2 and M3 acetonitrile–0.07% orthophosphoric acid (25:75, v/v). The high-performance liquid chromatographic assay allowed rapid, sensitive and reliable quantitation of verapamil and three of its metabolites in plasma without an extraction procedure. The limit of detection was less than 5 ng/ml (plasma) for all compounds. No interferences with other commonly co-administered drugs was observed. Plasma concentrations of verapamil and its metabolites were determined in 21 patients receiving a continuous infusion of verapamil for tachyarrhythmia of acute onset. The steady-state plasma concentration data of verapamil and its three main metabolites in these patients gave evidence that the plasma concentration of verapamil and its active metabolite norverapamil was primarily determined by the extent of the formation of M2.

INTRODUCTION

Racemic D,L-verapamil is a frequently used calcium antagonist for the treatment of atrial tachycardia, tachyarrhythmia or hypertension. The plasma half-life of verapamil ranges from 3 to 7 h [1–5]. Its plasma protein binding is of the order

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of 90% [1]. The oral bioavailability of verapamil is rather low (35%) with wide intra- and inter-individual variability due to an extensive first-pass metabolism [1–5]. Verapamil metabolism correlates with the extent of liver perfusion. Verapamil itself initially increases liver perfusion and thus leads to a transient increase in first-pass hepatic metabolism [3,5,6]. The therapeutic plasma concentration range has been established in different types of cardiovascular diseases [2,3,7,8]. Therapeutic plasma levels range from 60 to 300 ng/ml [1,4]. Verapamil is mainly metabolized to norverapamil (M1) by demethylation and to 2-(3,4-dimethoxyphenyl)-5-methylamino-2-isopropylvaleronitrile (M2, D 617) and 5-amino-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (M3, D 620) by oxidative dealkylation (Fig. 1) [9]. Norverapamil exerts about 20% of the vasodilator effect of verapamil, whereas M2 and its demethylation product M3 are inactive.

It is well known that rather different doses of verapamil are required for treating tachyarrhythmia in individuals [3,8]. The question remains whether the individual differences in verapamil dose requirements are due to individual differences in verapamil pharmacodynamics and/or individual differences in verapamil pharmacokinetics or its metabolism.

Several methods, including high-performance liquid chromatography (HPLC) [2–5,10–17], gas chromatography (GC) [1,18–20] and gas chromatography–mass spectrometry (GC–MS) [21–23], have been described for the determination of verapamil and its metabolites in plasma. For rapid determination of plasma concentrations of verapamil and its three main metabolites in patients with tachyarrhythmia, we developed an HPLC assay with fluorimetric detection.

EXPERIMENTAL

Reagents and chemicals

All chemicals, analytical grade or better, were used without further purification. Reference compounds of verapamil, norverapamil, M2 and M3 were a gift from Knoll (Ludwigshafen, Germany). The purity of the reference compounds was checked by GC–MS.

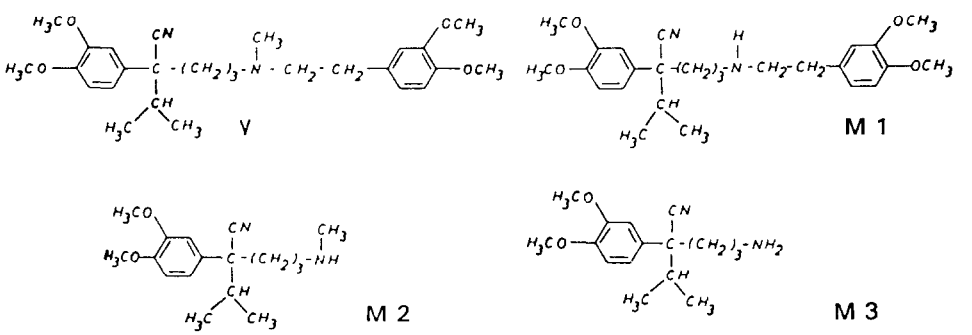

Fig. 1. Structure of verapamil (V), norverapamil (M1) and its two inactive metabolites, M2 and M3.

HPLC assay of verapamil and its metabolites

Verapamil and norverapamil (eluent A). A 0.2-ml sample of plasma was vortex-mixed with 0.3 ml of acetonitrile (Chrom, Mallinckrodt, St. Louis, MO, USA), allowed to stand for 10 min, vortex-mixed again and then centrifuged (3000 *g* for 5 min). A 20-μl aliquot of the supernatant was injected into the injection loop of the HPLC system. The HPLC system consisted of a Rheodyne 7125 (20-μl loop), a 410 pump, an LS-4 fluorescence detector and an LCI-100 computing integrator from Perkin Elmer (Bodenseewerk, Überlingen, Germany). The column was 125 mm × 4 mm I.D. with a precolumn (20 mm × 4 mm), both packed with Nucleosil C_{18}, 5 μm (Macherey & Nagel, Düren, Germany). For detection, an excitation wavelength of 280 nm and an emission wavelength of 313 nm were used. The eluent for separation of verapamil and norverapamil was a mixture of acetonitrile and 0.07% orthophosphoric acid (Merck, Darmstadt, Germany) (33:67, v/v). The flow-rate was 1.5 ml/min.

M2 and M3 (eluent B). For quantitation of M2 and M3, the supernatant of the deproteinated plasma (see above) was diluted with the same volume of 0.07% orthophosphoric acid. The eluent was a mixture of acetonitrile and 0.07% orthophosphoric acid (25:75, v/v). All other HPLC conditions were the same as described for verapamil and norverapamil.

Calibration and quantitation

Standard stock solutions were prepared in methanol and diluted with the mobile phase. The recovery was determined by comparison of the peak areas of verapamil and its metabolites from a stock solution with the peak areas of a pooled blank plasma spiked with verapamil, M1, M2 and M3 after the extraction procedure. For determination of the calibration curve, pooled blank plasma was spiked with increasing concentrations of verapamil and its metabolites (10, 50, 100, 200, 300, 400 and 600 ng/ml). The limit of detection was determined by spiking pooled blank plasma with decreasing concentrations of verapamil and its metabolites. For assessment of the day-to-day precision, plasma concentrations of verapamil and its metabolites were determined repeatedly on days 1, 3, 5 and 8. Plasma samples were stored at −20°C before analysis.

Clinical studies

After a loading dose of 2 × 5 mg verapamil intravenously (i.v.), 21 patients with tachyarrhythmia of acute onset received a continuous verapamil infusion of 2–20 mg/h (i.v.). The dose was adjusted to reduce the heart rate to at least 110/min. Venous blood was collected into heparinized tubes from all patients 24 h after constant verapamil infusion. Plasma was stored at −20°C before analysis. In all patients, laboratory findings indicated normal liver and kidney function. The antiarrhythmic efficacy of verapamil was assessed by electrocardiography and Holter monitoring.

RESULTS

HPLC assay

Owing to substantial differences in polarity, different eluents had to be used for the separation of verapamil and norverapamil (M1) and of M2 and M3. Gradient elution did not seem to be practical since a rather long column equilibration time was required. The retention times were 6.89 min (verapamil, eluent A), 6.40 min (norverapamil, eluent A), 4.89 min (M2, eluent B) and 4.40 min (M3, eluent B). The retention times of M2 and M3 were <2 min using eluent A and of verapamil and norverapamil >20 min using eluent B. Chromatograms of verapamil and norverapamil (eluent A) and M2 and M3 (eluent B) are depicted in Fig. 2.

The limit of detection for verapamil and its metabolites in plasma was 5 ng/ml. At plasma concentrations of 50 ng/ml, the coefficient of variation of replicate determinations of verapamil and its metabolites was 3–4%, the day-to-day precision 6%, and the recovery 98%. Reanalysis of plasma samples on days 3, 5 and 8 gave no evidence for a significant decay of plasma concentrations of verapamil and its metabolites, if plasma samples were stored at -20°C before analysis. The calibration curves were linear within a concentration range of 10–400 ng/ml for verapamil and its metabolites. The correlation coefficient was >0.991 for the calibration curves of verapamil and its three metabolites. No peaks interfering with the detection of verapamil, M1, M2 and M3 were observed in blank plasma. No interference of the assay with other commonly co-administered drugs such as glycerol trinitrate, isosorbide mononitrate, acetylsalicylic acid, lopirin, furosemide, digoxin or digitoxin was observed.

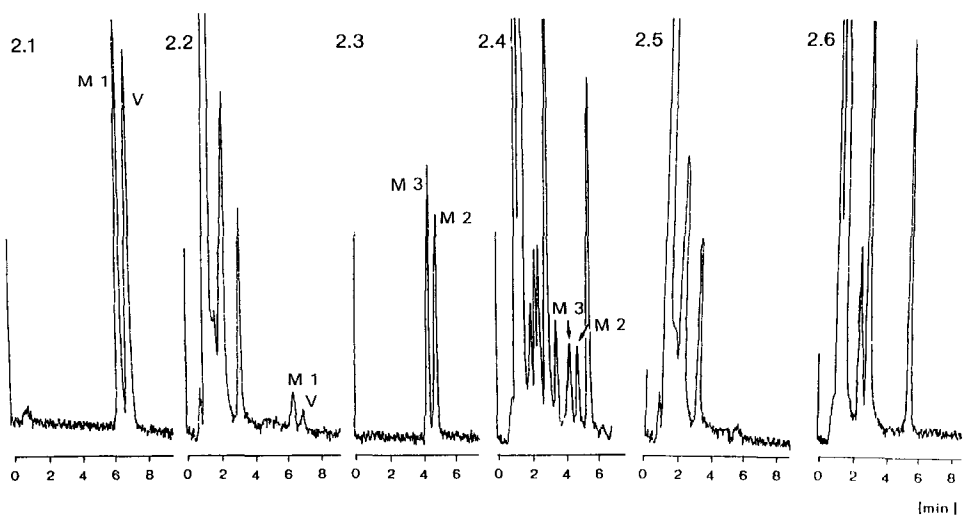

Fig. 2. HPLC profiles of a standard solution of 50 ng/ml verapamil (V) and 50 ng/ml norverapamil (M1) (2.1), a plasma sample from a patient (2.2), a standard solution of 50 ng/ml M2 and 50 ng/ml M3 (2.3), a plasma sample from a patient (2.4), and a blank plasma sample analysed with eluents A and B (2.5 and 2.6).

Patients

In all patients, a normofrequent sinus rhythm could be achieved by the verapamil infusion. The required verapamil doses showed a wide inter-individual range (2–20 mg/h). The same was true for plasma concentrations of verapamil (Fig. 3). Regression analysis revealed that the plasma concentration of verapamil inversely correlated with the plasma concentrations of M2 ($r = 0.92$) (Fig. 3). The same was true for the correlation of norverapamil plasma concentrations with the concentrations of M2 ($r = 0.89$). No correlation was found for the plasma concentrations of verapamil or norverapamil with that of M3. Plasma concentrations of verapamil and norverapamil were in a similar range in all patients.

DISCUSSION

The HPLC assay presented here allows routine determination of plasma concentrations of verapamil and its three main metabolites. The assay was sufficiently sensitive and reliable for pharmacokinetic studies. No interference of physiological substances or commonly co-administered drugs was observed. In contrast to the HPLC assays reported by other authors, an extraction procedure was not necessary for achieving high sensitivity [17,19].

Analysis of the verapamil plasma concentration data of the patients revealed that verapamil and norverapamil plasma concentrations inversely correlated with the plasma concentration of M2. This finding indicates that the plasma concentration of verapamil and its active metabolite norverapamil is primarily determined by the extent of formation of the metabolite M2. No correlation was found

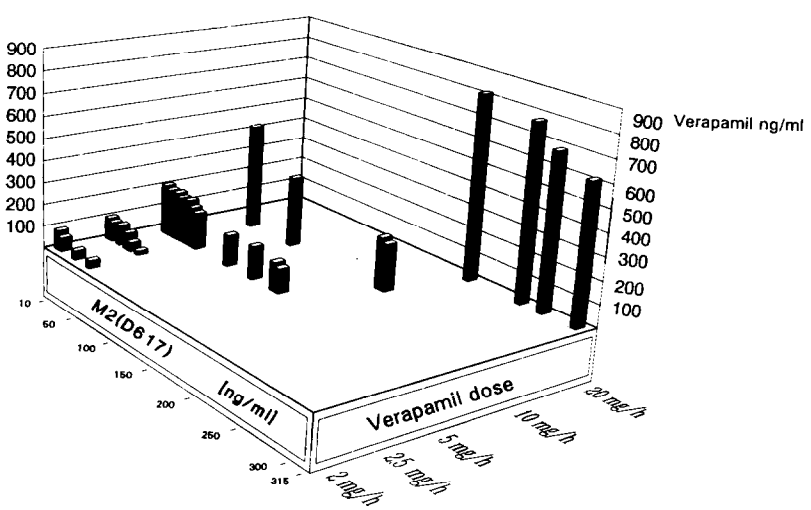

Fig. 3. Relationship between verapamil dose, plasma concentrations of verapamil and plasma concentrations of M2.

between the plasma concentration of verapamil or norverapamil and that of M3. The individual differences in the required verapamil dose obviously depend on individual differences in pharmacokinetics as well as on the pharmacodynamics of verapamil.

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