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Note

Quantification of nitrendipine in plasma by a capillary column gas chromatographic—mass spectrometric method

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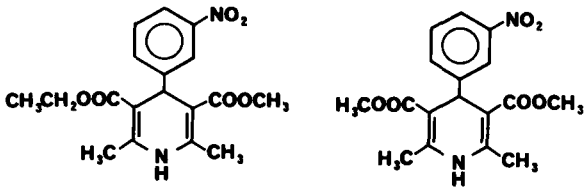
Calcium-blockers represent a group of drugs originally used for the treatment of angina pectoris, but in recent years also for hypertension [1, 2]. Nifedipine (Adalat®) was the first calcium-blocker of the dihydropyridine type. Although nifedipine has been found to effectively reduce the blood pressure of hypertensive patients [3], it has been difficult to find a significant correlation between the plasma concentration and the drop in blood pressure. This has been suggested to be due to the activation of compensatory mechanisms which may mask the "pure" effects of the drug [4]. Another reason may be that the analytical method predominantly used to determine nifedipine in plasma most likely includes an inactive metabolite [5].



I II

Fig. 1. Structures of nitrendipine (I) and the internal standard (II).

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Nitrendipine (I, Fig. 1) is a new calcium-blocker, structurally related to nifedipine. Pharmacological studies indicate that nitrendipine is a potent calcium-blocker, probably slower in onset of action, but more potent and with longer antihypertensive action than nifedipine [6, 7]. Recent studies indicate that nitrendipine may not trigger the compensatory mechanisms (causing, for example, elevated heart rate) to the same extent as nifedipine [8]. This increases the possibilities of finding a correlation between the blood pressure reducing effect and plasma concentration.

Prior to performing a pharmacokinetic study of nitrendipine, the following capillary column gas chromatographic—mass spectrometric (GC—MS) method was developed to ensure high sensitivity and specificity of the assays.

EXPERIMENTAL

Chemicals

Nitrendipine (I) and the structural analogue used as internal standard, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester (II, Fig. 1), were obtained from Bayer (Wuppertal, F.R.G.). Stock solutions were prepared (approx. 1 mg/ml) in 20% methanol and stored in the dark at 4°C. Other chemicals used were of analytical purity and were obtained from commercial sources.

Plasma samples

The plasma samples were obtained from subjects suffering from essential hypertension of mild to moderate severity. The subjects received a single oral dose of 20 or 40 mg of nitrendipine, after which the plasma samples were drawn. A blood sample 24 h after administration was also obtained after three weeks' treatment. The plasma samples were protected from light and stored at −70°C prior to analysis.

Sample preparation

Aliquots of plasma (1.0 ml) were pipetted into 15-ml glass-stoppered test-tubes containing 79.2 pmol of the internal standard (II). Following the addition of 1.0 ml of 0.1 *M* sodium hydroxide and 2.5 ml of diethyl ether, the samples were shaken and centrifuged at 1000 *g* for 5 min. The diethyl ether layers were transferred to new test-tubes and evaporated to dryness under a stream of nitrogen. The residues were redissolved in 50 μl of ethyl acetate.

Gas chromatography—mass spectrometry

An LKB 2091 gas chromatograph—mass spectrometer was used for the analyses of plasma extracts and recording of mass spectra.

The GC separations were achieved using a 10 m × 0.32 mm I.D. WCOT SE-52 glass capillary column. Helium was used as a carrier and make-up gas. Splitless injections were carried out using a "moving needle" device. The GC conditions were: injector heater 300°C, column temperature 225°C, column flow-rate approx. 2 ml/min, and make-up gas flow-rate approx. 12 ml/min. Aliquots of 1 μl of the plasma extracts were injected and an initial delay of 0.5 min in opening the separator valve was used to avoid contamination of

the ion source. Under these conditions the retention time of nitrendipine and the internal standard was about 2 min. The MS conditions were: separator temperature 275°C, ion source temperature 240°C, electron energy 70 eV, and trap current 50 μA.

Quantification

Standard samples were prepared by spiking blank serum with nitrendipine (0—72.8 ng/ml). Calibration curves were constructed by plotting the peak height ratio nitrendipine/internal standard obtained in the GC—MS analyses against the concentration of nitrendipine. The nitrendipine levels were then

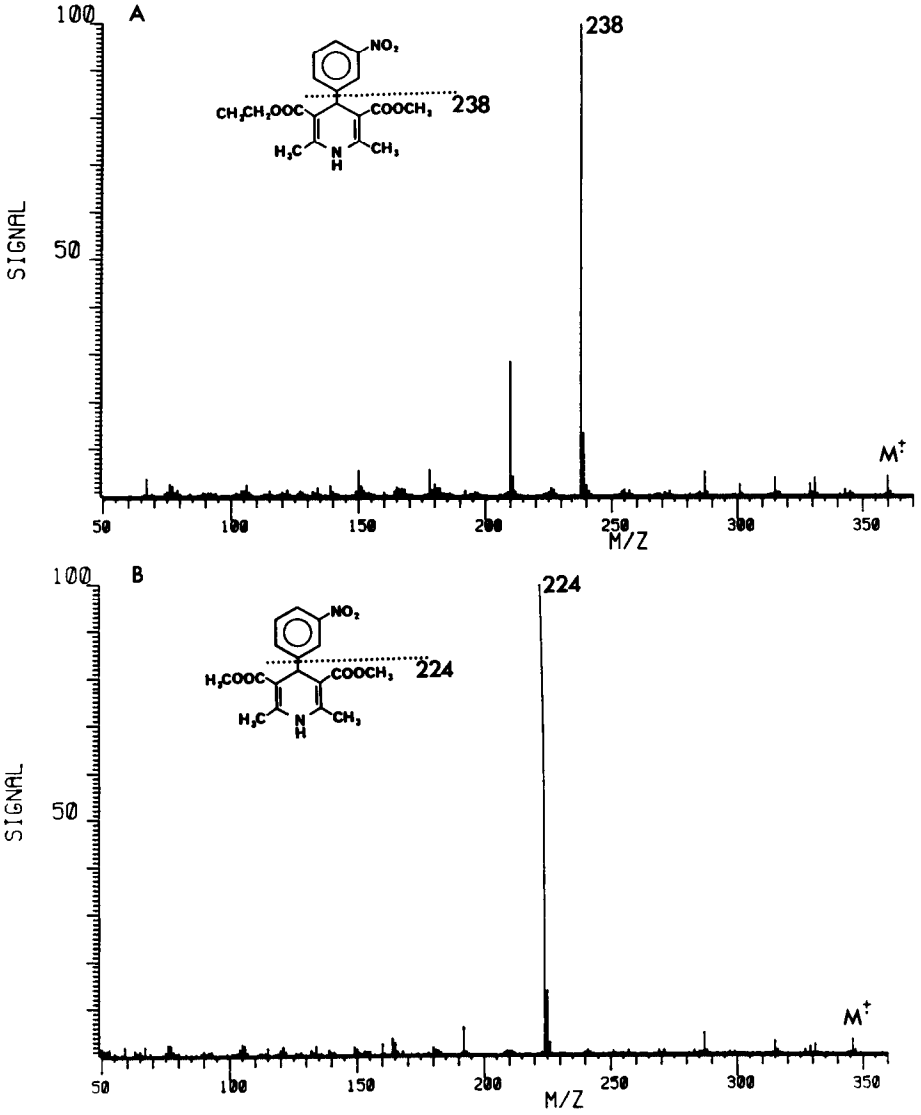

Fig. 2. Electron-impact mass spectra of (A) nitrendipine (I), and (B) the internal standard (II).

determined from the peak height ratio of each plasma extract by reference to the calibration curve.

RESULTS AND DISCUSSION

The electron-impact mass spectra of nitrendipine and the structure analogue (II) chosen as internal standard revealed molecular ions at *m/z* 360 and 346, respectively, which were of low intensity (Fig. 2). The base peaks at *m/z* 238 and 224 were formed by elimination of the 3-nitrophenyl moieties. These mass numbers were monitored in the ion-specific analysis of the plasma extracts.

A representative ion trace chromatogram obtained from the analysis of a plasma sample is shown in Fig. 3. The precision of the quantitative determinations was estimated by repeated analysis of two pools of plasma which had been spiked with nitrendipine (3.64 and 36.4 ng/ml), and was found to be better than 5%. The peak height ratios nitrendipine/internal standard were found to possess a linear relationship to the concentration of nitrendipine in the standard samples in the range of 0—72.8 ng/ml. The yield of nitrendipine and the internal standard through the sample preparation procedure was estimated to be greater than 90%. No evidence for a conversion of the internal standard to nitrendipine or vice versa was recorded under the conditions employed in the analytical procedure. The method was capable of determining nitrendepine levels below 0.5 ng/ml of plasma.

The method was used to determine the plasma concentrations after oral intake of 20 mg or 40 mg of nitrendipine (Fig. 4). The plasma concentration of nitrendipine peaked approximately 90 min after the administration of both 20

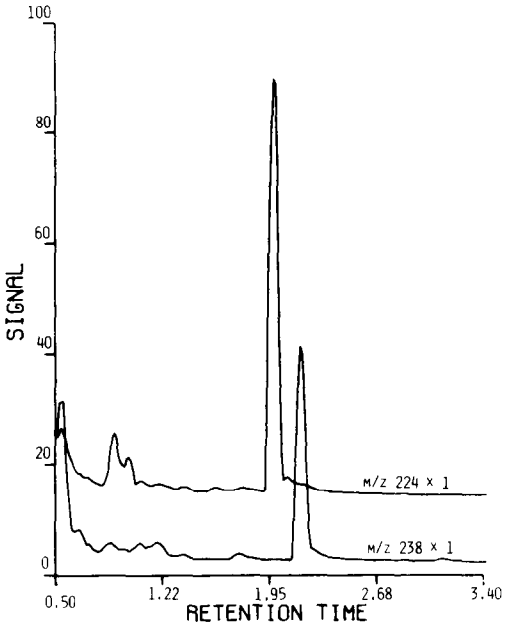

Fig. 3. Chromatogram obtained from the analysis of a plasma sample. The mass numbers (*m/z*) and relative amplification factors are indicated. The retention time is expressed in min.

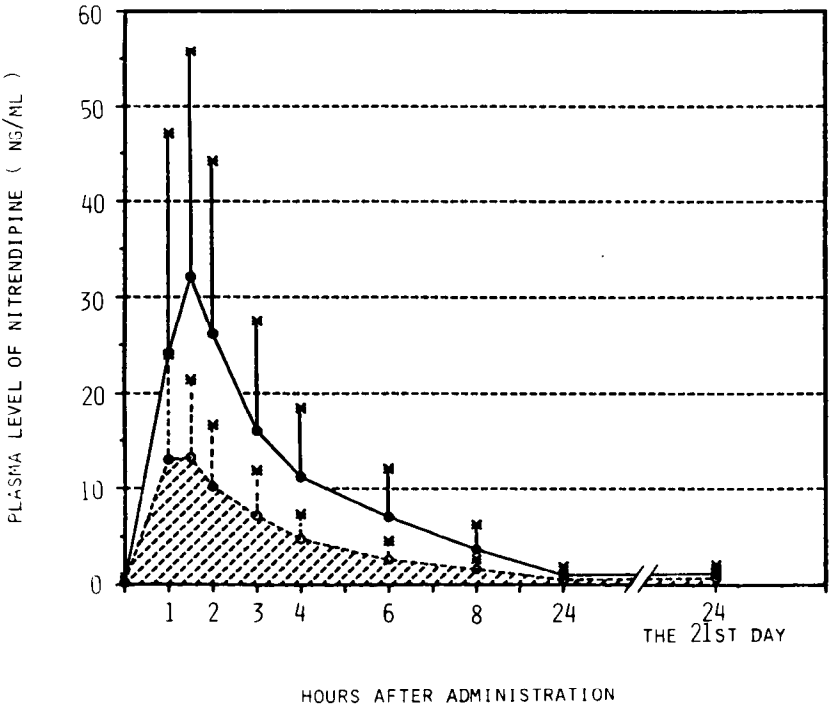

Fig. 4. Time course of nitrendipine levels in human plasma after oral intake or 20 (○) or 40 (●) mg.

and 40 mg. Despite the fact that the area under the curve was nearly twice as big on 40 mg [144.2 (ng/ml) × h] as on 20 mg [69.6 (ng/ml) × h], no additional blood pressure reduction was obtained, indicating that the maximum effect can be reached with 20 mg.

The results of the pharmacokinetic and pharmacodynamic analysis also revealed that the β-elimination half-life, i.e. the half-life of nitrendipine in plasma during the elimination phase, was 11.4 h on 20 mg and 8.1 h on 40 mg, and furthermore that there was a significant correlation between the plasma concentration of nitrendipine and blood pressure lowering effect (20 mg: $r = 0.72, p < 0.05$; 40 mg: $r = 0.84, p < 0.001$) [8].

In conclusion, a highly sensitive and specific method for the analysis of nitrendipine in plasma was developed. The precision and specificity of the analysis have made it possible to demonstrate a correlation between plasma levels and decrease in blood pressure for these types of drugs. This fact emphasizes the importance of using reliable analytical methods when performing pharmacokinetic research.

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