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Note

Gas chromatographic analysis of nitrendipine and its pyridine metabolite in human plasma

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Nitrendipine [3-ethyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, Fig. 1], a potent dihydropyridine calcium entry blocker, is currently undergoing clinical trials for the treatment of hypertension [1].

Analytical methods already published for the quantification of nitrendipine in biological fluids include gas chromatography (GC) with mass spectrometric (MS) detection [2, 3] and with electron-capture detection (ECD) [4, 5], liquid chromatography (LC) with UV detection [5, 6], radioreceptor assays [6–8] and radioimmunoassays [9, 10].

The concentrations of nitrendipine in human plasma are relatively low as a consequence of its high pharmacological potency [5]. The LC methods [5, 6] therefore are not sensitive enough for detailed pharmacokinetic studies. GC–MS [2, 3] is very sensitive but requires expensive instrumentation, whereas one of the GC methods using ECD [5] is non-specific as a consequence of thermal deg-

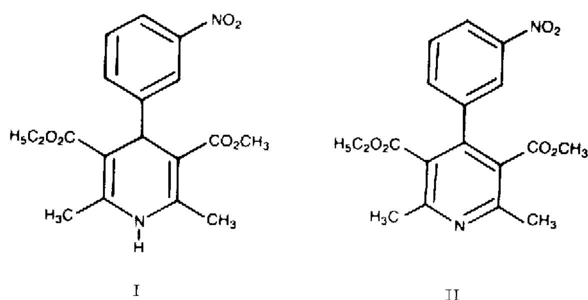

Fig 1 Structures of nitrendipine (I) and its pyridine metabolite (II).

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radation in the injector system, so that the corresponding pyridine derivative, which is known to be one of the metabolites in humans, is co-determined. The radioreceptor assays [6–8] are unable to detect the pharmacologically inactive metabolites that lack the dihydropyridine structure. This is a disadvantage when studying the metabolism of nitrendipine. For the radioimmunoassays [9, 10] unwanted cross-reactivity may represent a problem, making the assay non-specific.

Because of some of the shortcomings of the published methods we developed a simple, specific and sensitive GC assay with ECD for nitrendipine and its pyridine metabolite (Fig. 1), which is suitable for measurements in the sub-nanogram per millilitre range. This is required for single-dose pharmacokinetic studies.

EXPERIMENTAL

Chemicals and glassware

Nitrendipine, its pyridine metabolite and the internal standard, 3-(2-hydroxy-2-methyl)ethyl-5-methyl-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylate, were kindly supplied by Bayer (Wuppertal, F.R.G.). Solutions of these substances at suitable concentrations were made in distilled methanol p.a. All the other chemicals used were of analytical grade (Baker, Deventer, The Netherlands). The organic solvents were always distilled twice prior to use. All glassware was rinsed with methanol p.a. and dried at 250°C for at least 2 h.

Apparatus and chromatographic system

A Hewlett-Packard Model 5890A gas chromatograph, equipped with a ^{63}Ni pulse-modified electron-capture detector and a laboratory-made all-glass solid injection system with a moving needle were used. A fused-silica capillary column (30 m×0.32 mm I.D.) with a DB-1 (bonded cross-linked silicon) stationary phase (Durabond, J & W Scientific, Rancho Cordova, CA, U.S.A.) was used.

The operating conditions were: injection port temperature, 260°C; detector temperature, 350°C; column temperature, 245°C for 6.4 min, then rising to 270°C (40°C/min) and remaining at that temperature for 2.0 min. After cooling to 245°C, a stabilization time of at least 2 min was allowed. The carrier gas was helium (Hoek-Loos, Schiedam, The Netherlands); flow-rates were 2.0 ml/min through the column and 15 ml/min through the restrictor of the injector system. The flow-rate of the auxiliary gas (argon–methane, 95:5) was 30 ml/min.

Data were processed with a Hewlett-Packard 3392A integrator in peak-height mode.

Extraction procedure

To 1.00 ml of plasma in a centrifuge tube, 0.99 ng of internal standard was added. After mixing and equilibrating for at least 10 min, 25 μl of 4 *M* sodium hydroxide were added and mixed. The sample was extracted with 4.0 ml of *n*-pentane–dichloromethane (7:3) for 20 min on a whirl-mixer [Vortex vacuum evaporator (vacuum mode off), Buchler, Fort Lee, NJ, U.S.A.]. After centrifugation for 5 min at 2000 *g*, the upper organic layer was transferred to another

centrifuge tube using Pasteur disposable pipettes and evaporated to dryness on the same vortex vacuum evaporator at 35°C.

The residue was dissolved in 50 μl of n-pentane–dichloromethane (7:3), and 2.0 μl were brought on the tip of the glass needle of the solid injector system. The solvent was allowed to evaporate for 35 s, and the residue was injected into the gas chromatograph.

Because other dihydropyridine compounds, such as nifedipine [11] and nisoldipine [12], are known to be very sensitie to light, and no reliable data on the photostability of nitrendipine were available at the time of this study, the extraction procedure was carried out under sodium light, with daylight excluded.

Preparation of calibration curves

Calibration curves were prepared by spiking control plasma samples (1.00 ml) with nitrendipine (0, 0.0993, 0.248, 0.496, 0.744, 0.993, 2.48, 4.96, 9.93 and 19.9 ng/ml) and its pyridine metabolite (0, 0.101, 0.254, 0.507, 0.761, 1.01, 2.54, 5.07, 10.1 and 20.3 ng/ml). Samples were processed as described, and the ratios of the peak height of the compound to be assayed to the peak height of the internal standard were calculated. Calibration curves were constructed by linear regression analysis.

Extraction yield and reproducibility

The extraction yield of the internal standard (0.993 ng/ml, $n=10$), nitrendipine and its pyridine metabolite (both in the range 0.05–20 ng/ml, $n=18$) were determined on three different occasions with the internal–external standard method. The intra-assay variability was determined at two concentration levels (0.1 and 1.0 ng/ml) on two occasions (for each concentration on each occasion, $n=4$). The inter-assay variability was determined at different concentration levels in the range 0.1–20 ng/ml on at least seven occasions.

Human experiments

The assay was used to study the pharmacokinetics of nitrendipine in nine healthy male volunteers after intravenous administration of 40 μg/kg body mass. Blood samples (8 ml) were drawn from a forearm vein using a flexible canula (Venflon®) or by vena punction. Samples were drawn in heparinized tubes (Vacutainer®) at 0, 10, 20, 30, 45, 60, 75, 90 and 105 min and 2, 3, 4, 6, 8, 12, 24, 32 and 48 h after drug administration. Plasma was separated immediately by centrifugation and samples were stored at −30°C until analysed.

RESULTS

Assay of nitrendipine and its pyridine metabolite in plasma

Fig. 2 shows typical chromatograms obtained after extraction of blank plasma, blank plasma to which only internal standard had been added and plasma containing nitrendipine and its pyridine metabolite. Although several substances seem to be co-extracted, none interferes significantly with the assay of nitrendipine and its pyridine metabolite. Under these conditions no thermodegradation

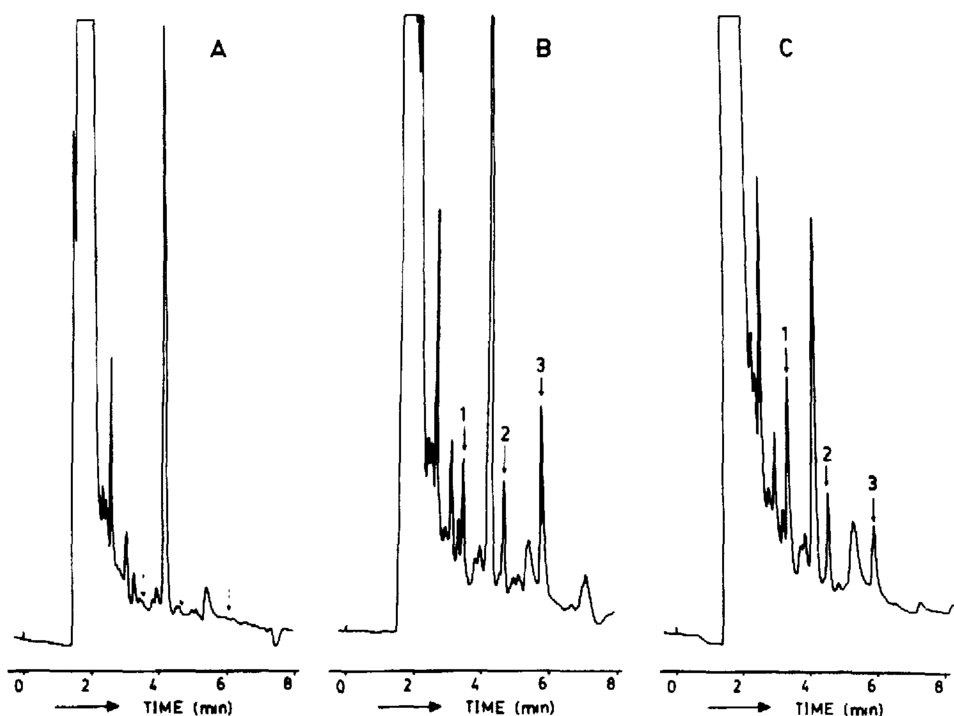

Fig. 2. Chromatograms of (A) control plasma, (B) plasma 2 h after intravenous administration of nitrendipine (40 μg/kg), containing 2.38 ng/ml nitrendipine (peak 3), 0.99 ng/ml internal standard (peak 2) and 0.53 ng/ml pyridine metabolite (peak 1) and (C) control plasma spiked with 0.99 ng/ml nitrendipine (peak 3), with 0.99 ng/ml internal standard (peak 2) and with 1.01 ng/ml pyridine metabolite (peak 1).

of nitrendipine was observed. The pyridine metabolite, the internal standard and nitrendipine had retention times of 3.3, 4.5 and 5.8 min, respectively. The total run time was ca. 11 min.

Calibration curves for nitrendipine were always linear with a coefficient of correlation of at least 0.998, and the intercept was not different from zero. The calibration curves for the pyridine metabolite were usually linear with a coefficient of correlation of at least 0.998, but for unknown reasons they were sometimes best described as being composed of two straight lines, with the smaller slope at higher concentrations, and with a small intercept. The extraction yields were (mean ± S.D.): internal standard, 87±7% (n=10); nitrendipine, 91±9% (n=18); pyridine metabolite, 75±9% (n=18).

The intra-assay coefficient of variation (C.V.) for nitrendipine was 8.9% at 0.0993 ng/ml and 4.5% at 0.993 ng/ml. For the pyridine metabolite the intra-assay C.V. was 7.7% at 0.101 ng/ml and 3.4% at 1.01 ng/ml. The inter-assay C.V. varied from 9.1% at 0.1 ng/ml to 4.9% at 20 ng/ml (n=7–19).

Arbitrarily, it can be concluded that the lowest concentration that could be quantified reliably of each compound was ca. 0.05 ng/ml, depending on the quality of the plasma.

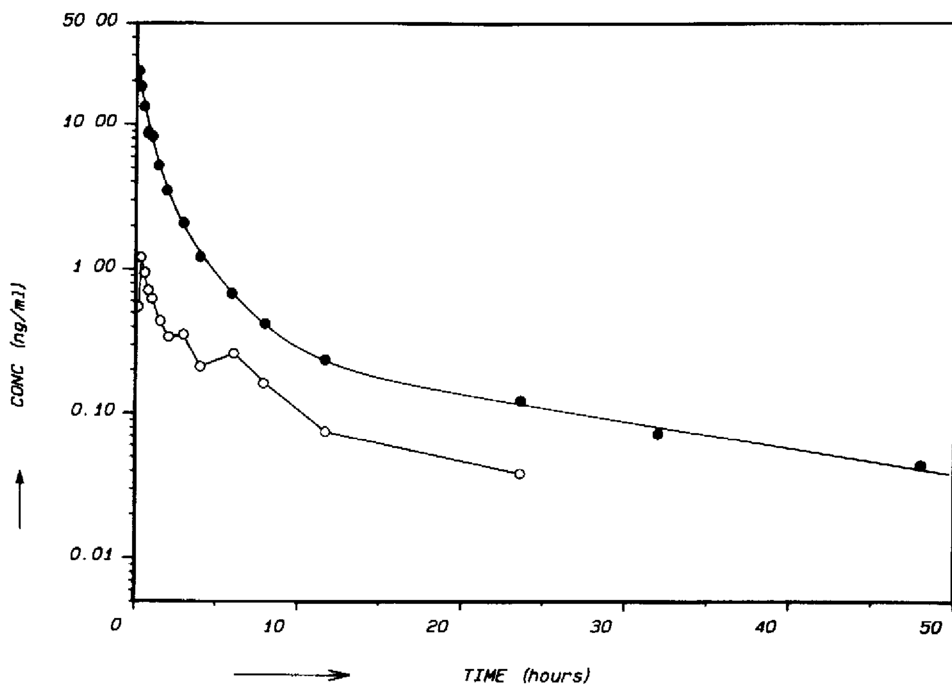

Fig. 3. Plasma concentration–time profile of nitrendipine (●) and its pyridine metabolite (○) after intravenous administration of 40 μg/kg nitrendipine.

Human experiments

Representative plasma concentration–time profiles for both compounds are shown in Fig. 3. From these data the terminal elimination half-life of 11.7 ± 5.4 h (mean ± S.D.) for nitrendipine was calculated by logarithmic regression analysis. The systemic clearance, calculated as the dose divided by the area under the curve (AUC) extrapolated to infinity, was 1.47 ± 0.22 l/min.

DISCUSSION

This paper describes a sensitive but rather simple GC method for the simultaneous analysis of nitrendipine and its pyridine metabolite in human plasma. For some other dihydropyridine calcium entry blockers, sensitive (capillary) GC methods have already been published, most of them using ECD [12–22] or MS detection [23, 24]. Although usually very specific, the LC assays with UV detection for the other dihydropyridine calcium entry blockers [5, 6, 11, 14, 19, 25–28], are not suitable for single-dose pharmacokinetic studies with nitrendipine, because of the lack of sensitivity. This also holds for the LC methods with electrochemical detection [29–31].

The present method is sensitive (down to 0.05 ng/ml), specific and reproducible (inter-assay variability 4.9–9.1%), making the method useful for single-dose pharmacokinetic studies. In this low concentration range, the quality and purity of the carrier gas, the make-up gas and the organic solvents were of great

importance. Our glassware had to be rinsed with methanol p.a. prior to use. Despite these time-consuming precautions, up to 40 samples (including calibration curves) could be processed per day.

The only assays for nitrendipine published so far with a comparable sensitivity are the radioimmunoassay of Campbell et al. [9], which, however, does not allow the quantification of the pyridine metabolite. The GC method of Fischer et al. [3] is also very sensitive, but it requires the rather complicated electron-capture negative-ion chemical ionization MS detection. The GC–ECD of Kann et al. [4] is unfortunately not very well documented.

The present method was used for single-dose pharmacokinetic studies with nitrendipine. With some modifications in the extraction procedure and the column temperature, we also used this method for analysing nifedipine and its pyridine metabolite in single-dose pharmacokinetic studies, with a limit of detection of ca. 0.08 ng/ml, using 0.5-ml plasma samples.

CONCLUSIONS

The GC method described in this paper is rapid, selective and sensitive enough for use in single-dose pharmacokinetic studies on nitrendipine in humans. It allows the simultaneous determination of nitrendipine and its pyridine metabolite down to 0.05 ng/ml in plasma. The method is simple, and requires neither complicated instrumentation nor the isolation of receptors or antibodies, and no open sources of radioactivity are needed. With some modifications this method could also be used to analyse other dihydropyridine calcium entry blockers in human plasma.

ACKNOWLEDGEMENT

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NOTE

After completion of this manuscript we became aware of another recently published GC method: G.J. Krol, J.T. Lettieri, A.L. Mazzu, D.E. Burkholder, J.P. Birkett, R.J. Taylor and C. Bon, J. Cardiovasc. Pharmacol., 9 (Supp. 4) (1987) S129.

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