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Note

Gas chromatographic determination of nisoldipine and one of its metabolites in plasma

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Nisoldipine, isobutylmethyl-1,4-dihydro-2,6-dimethyl-4- (2-nitrophenyl) pyridine-3,5-carboxylate (Fig. 1), is a potent calcium channel blocker, which selectively seems to dilate arterioles with little or no effect on other blood vessels or the heart [1]. Therefore, the compound is presently being developed for the use in the treatment of angina pectoris [2, 3] and hypertension [4, 5].

For the determination of nisoldipine in biological fluids no methods have been published yet, probably because of the very low therapeutic concentration range in human plasma. Methods for the determination of similar dihydropyridine calcium channel blockers (nifedipine, nicardipine, nimodipine, nitrendipine, ryosidine and felodipine) in biological fluids include gas chromatography (GC) with mass spectrometric (MS) detection [6], electron-capture detection [7–16], nitrogen detection [17] or flame-ionization detection [18], and liquid chromato-

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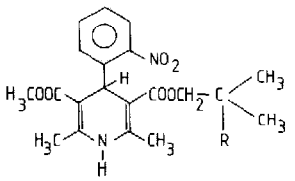

R = H Nisoldipine
R = OH Metabolite I

Fig. 1. Structures of nisoldipine (Bay k 5552) and metabolite I.

graphy with spectrophotometric detection [19, 20] and electrochemical detection [21]. A radio-receptor assay for nitrendipine in serum has been published [19].

When using GC, one of the major problems is the oxidation of dihydropyridines to their corresponding pyridine derivatives in the injector and the column at high temperatures (230–270°C). Therefore, some investigators [10, 13] have oxidized the dihydropyridines and determined the corresponding pyridine compounds. A major drawback of this approach is the fact that the pyridine derivative is not only a conversion product, but also a metabolite, making the assay non-specific. Other authors determined unchanged dihydropyridines, using both packed-column [8, 9, 11, 12] and (fused-silica) capillary column [7, 15–17] GC.

Because of the great sensitivity to light of 2-nitrophenyldihydropyridines [16, 20, 22, 23], special precautions in handling the drug during intravenous therapy and its analysis in plasma are necessary.

In the present study a sensitive and selective GC method has been developed for the determination of unchanged nisoldipine and its side-chain hydroxylated metabolite I (Fig. 1) in plasma, taking into account possible photodecomposition. Preliminary disposition data in healthy volunteers are included. In addition, the photostability of nisoldipine in organic solvents, as well as in plasma, was investigated.

EXPERIMENTAL

Chemicals

Nisoldipine, its 2-hydroxyisobutyl metabolite and nitrendipine were kindly supplied by Bayer (Wuppertal, F.R.G.). In all experiments freshly distilled organic solvents (Baker, Deventer, The Netherlands) were used.

Apparatus and chromatographic system

A Hewlett-Packard Model 5890A gas chromatograph, equipped with a ^{63}Ni pulse-modified electron-capture detector and a tailor-made all-glass solid injection system was used. A cross-linked fused-silica capillary column (30 m × 0.326 mm I.D.) with a DB-1 stationary phase (Durabond, J & W Scientific, Rancho Cordova, CA, U.S.A.) was used. The operating conditions were: injection port temperature, 260°C; column temperature, 250°C; detector temperature, 350°C; flow-rate of helium carrier gas, 2.5 ml/min; flow-rate of auxiliary gas (ar-

gon–methane, 95:5), 25 ml/min; helium flow-rate through the restrictor in the injector, 15 ml/min. Under these conditions the internal standard (nitrendipine), nisoldipine and metabolite I had retention times of 6.4, 6.7 and 8.9 min, respectively. Data were processed using a Shimadzu C-R3A integrator (peak-height mode).

Mass spectra for identification of eluted substances were taken on an LKB 2091-2130 gas chromatograph–mass spectrometer equipped with a Digitial PDP 11/05 computer under similar chromatographic conditions to those applied in the assay procedure.

Extraction procedure

To 1 ml of plasma in a centrifuge tube 0.27 ng nitrendipine (internal standard) in methanol was added. After mixing and equilibrating for 15 min 25 μl of 6 *M* sodium hydroxide were added. After homogenization the sample was extracted with 3 ml of pentane–dichloromethane (7:3) for 30 min on a Sarstedt CM-9 (Sarstedt, Nümbrecht, F.R.G.) whirlmixer. After centrifugation (10 min at 2000 *g*) the upper organic layer was transferred to a 10-ml centrifuge tube using Pasteur disposable pipettes, and evaporated to dryness on a vortex vacuum evaporator (Buchler, Fort Lee, NJ, U.S.A.) at 40°C. Finally, the residue was dissolved in 50 μl of dichloromethane, and 2 μl of this solution were brought onto the glass needle of the solid injection system. After evaporation of dichloromethane, the residue was injected into the gas chromatograph. All steps were carried out in sodium light, excluding daylight.

The influence of the duration of whirlmixing on the extraction yield was determined by mixing for 0, 5, 10, 20, 30, 45 and 60 min and subsequent extraction. Extraction yields of nisoldipine and metabolite I were determined using the internal/external standardization method for the compound under investigation.

Preparation of calibration graphs

Control plasma samples (1.0 ml) were spiked with 0, 0.12, 0.24, 0.35, 0.47, 0.59, 1.18, 2.35 and 4.70 ng/ml nisoldipine and 0, 0.15, 0.30, 0.45, 0.60, 0.74, 1.49, 2.98 and 5.95 ng/ml metabolite I. Samples were processed as described and the ratios of the peak height of the compound to be assayed to the peak height of internal standard were calculated. Calibration curves were constructed by linear regression analysis.

Photostability

Nisoldipine (50 ng) and metabolite I (50 ng) dissolved in 5 ml of dichloromethane–*n*-pentane (3:7) in a 10-ml centrifuge tube were exposed to normal laboratory daylight on a day in June for 0, 20, 40, 60 and 90 min. After exposure to light, internal standard was added and the samples were directly analysed by GC. The same procedure was performed after spiking plasma samples with nisoldipine and metabolite I (10 ng/ml) and exposing these to light. These samples were extracted prior to analysis.

Human experiments

Six healthy young men received 10 mg of nisoldipine orally in an aqueous solution. Blood samples (5–10 ml) were taken from a forearm vein using a flexible

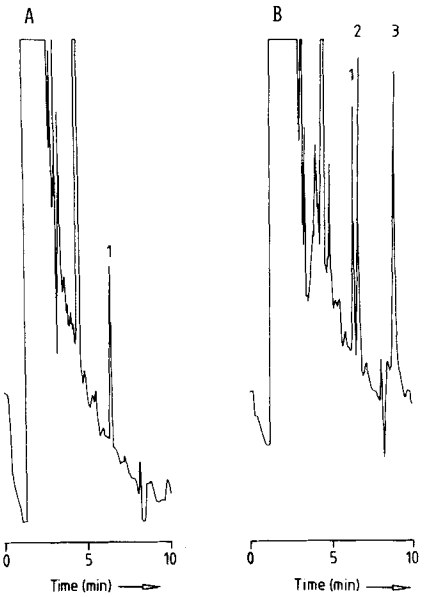

Fig. 2. Chromatograms of (A) control plasma with internal standard (peak 1) and (B) plasma spiked with internal standard (peak 1), 1.18 ng/ml nisoldipine (peak 2) and 1.49 ng/ml metabolite I (peak 3).

cannula (Venflon®). Samples were drawn in heparinized tubes (Vacutainer®) at 0, 10, 20, 30, 40, 50, 60, 75 and 90 min and 2, 3, 4, 5, 6, 8 and 10 h after drug administration. Plasma was separated by centrifugation and samples were stored (protected from light) at −30°C until analysed.

RESULTS AND DISCUSSION

Assay of nisoldipine and metabolite I in plasma

Fig. 2 shows typical chromatograms obtained after extraction of blank plasma to which only internal standard had been added and of plasma containing 1.18 ng/ml nisoldipine and 1.49 ng/ml metabolite I. No interfering substances seemed to be coextracted from plasma and no interfering photo- or thermodegradation products were seen. MS demonstrated that both nisoldipine and metabolite I were determined unchanged.

Calibration curves were linear with concentration, as shown by the correlation coefficient (minimally 0.999) and the intercept, which was not different from zero. The method was reproducible and had satisfactory precision: standard deviations never exceeded 10% in the concentration range 0.1–50 ng/ml. The limit of detection for both nisoldipine and metabolite I was ca. 50 pg/ml, depending on the quality of the plasma.

After whirlmixing for 30 min, the extraction yield proved to be maximal for nisoldipine, with a value of 78.5 ± 1.2 % (mean ± S.D., $n=4$). After 30 min the extraction yield of metabolite I was 92.8 ± 3.5% ($n=4$), although complete extraction was achieved after 10 min of whirlmixing.

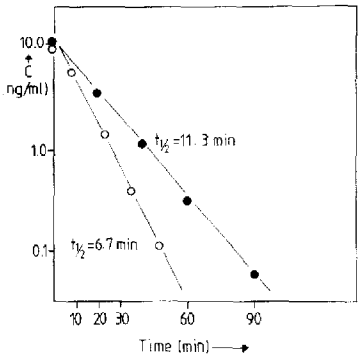

Fig. 3. Decomposition of nisoldipine exposed to daylight in organic solvent (open circles) and plasma (closed circles).

Photostability

Nisoldipine and metabolite I proved to be extremely photolabile compounds, both in organic solvents and in plasma (Fig. 3). In June, the decomposition half-life of nisoldipine was 6.7 min in dichloromethane–pentane (3:7) and 11.3 min in plasma. For metabolite I the values were 6.3 min and 10.7 min, respectively, indicating that the whole assay procedure has to be carried out strictly out of daylight. In sodium light no photodestruction could be detected after two weeks.

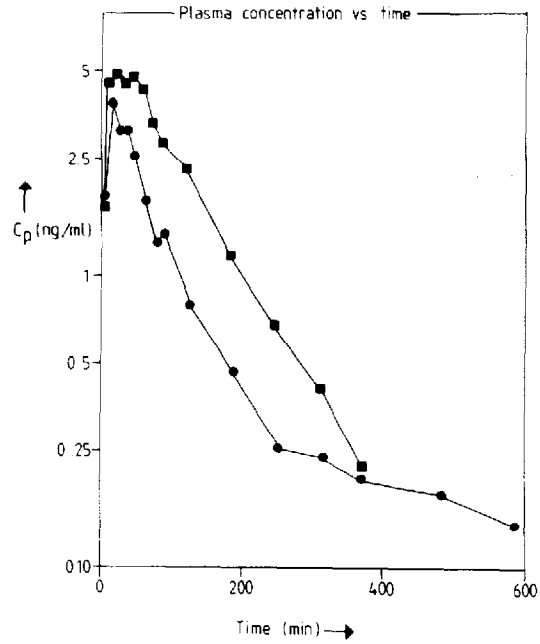

Fig. 4. Plasma concentration–time profiles of nisoldipine (circles) and metabolite I (squares) in a healthy subject (subject C) after oral ingestion of 10 mg of nisoldipine in solution.

TABLE I

PHARMACOKINETIC DATA OF NISOLDIPINE IN SIX HEALTHY SUBJECTS AFTER ORAL INGESTION OF 10 mg OF NISOLDIPINE IN AQUEOUS SOLUTION

$t_{1/2}$ = elimination half-life; Cl_{or} = clearance obtained by dividing dose by AUC; C_{max} = maximal plasma concentration.

Subject	Age (years)	Weight (kg)	Nisoldipine			Metabolite I	
			$t_{1/2}$ (h)	Cl_{or} (l/min)	C_{max} (ng/ml)	$t_{1/2}$ (h)	C_{max} (ng/ml)
C	42	64	6.6	22	3.9	1.2	5.0
E	35	69	3.7	61	1.5	1.2	4.8
J	24	79	3.1	19	5.7	1.6	8.4
H	30	68	3.5	7	17.6	2.3	17.7
K	34	89	3.3	29	7.4	1.7	10.6
G	27	80	2.4	75	1.8	1.3	6.2
Mean	32	75	3.7	35	6.3	1.5	8.8
S.D	6	9	1.5	27	6.0	0.4	4.9

Human experiments

Representative plasma concentration–time curves for both compounds are shown in Fig. 4. From these data, the elimination half-life was calculated by linear regression analysis of the terminal parts of the curves, and the oral clearance by dividing dose by area-under-the-curve (AUC) extrapolated to infinity [24]. Individual parameters are given in Table I. After oral administration of 10 mg (solution) to six healthy male volunteers (age 32 ± 6 years, weight 75 ± 9 kg), the terminal plasma half-life proved to be 3.7 ± 1.5 h. Oral clearance was very high and differed very much between individuals (35 ± 27 l/min; range 7–75 l/min), indicating very rapid metabolism (high first-pass effect). Maximal levels of nisoldipine were 6.3 ± 6.0 ng/ml. Terminal plasma half-life of metabolite I was 1.5 ± 0.4 h; maximal plasma concentrations were 8.8 ± 4.9 ng/ml.

CONCLUSIONS

The GC method described in this paper for the determination of both nisoldipine and its side-chain hydroxylated metabolite in plasma allows their rapid, sensitive and selective quantitative analysis without interference from endogenous compounds, metabolites or photodegradation products. The method is suitable for pharmacokinetic studies in humans. It is clearly shown in the present study that nisoldipine plasma samples need to be handled strictly out of daylight.

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