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Note

Determination of nimodipine by gas chromatography using electron-capture detection; external factors influencing nimodipine concentrations during intravenous administration

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Nimodipine, isopropyl 2-methoxyethyl 1,4-dihydroxy-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate, is a vasorelaxant dihydropyridine derivative with a calcium antagonistic mechanism of action and a preferential effect on cerebral vessels [1—5] — clinical studies indicate that this calcium antagonist is effective in the therapy of cerebrovascular spasms of various origins. The drug seems to be especially effective in prevention of cerebral arterial spasms in patients with subarachnoid haemorrhagia from intracranial aneurysms [6, 7].

To prevent cerebrovascular spasms after surgery, patients had to be treated several days after operation with continuous intravenous infusion of nimodipine; however, administration of dihydropyridines is connected with some problems, as it has been shown that some of these drugs are very sensitive to light [8].

Krol et al. [9] have described a method for determination of nimodipine by gas chromatography (GC). In their study, nimodipine is converted by oxidation to the more stable pyridine analogue, which is, however, also formed in vivo by metabolism of nimodipine, so only the sum of the two compounds is

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determined. The present paper describes a sensitive, simple, rapid and selective method for the determination of nimodipine by GC.

Preliminary studies in our laboratory have shown that nimodipine is absorbed to polyvinyl chloride (PVC). In order to evaluate absorption to infusion sets and the possible influence of light during continuous intravenous administration of nimodipine, the concentration of nimodipine in solutions and in blood plasma was determined.

EXPERIMENTAL

Standards and reagents

Nimodipine and nitrendipine (internal standard) in pure crystalline form and a solution of nimodipine (0.2 mg/ml) for intravenous administration were kindly supplied by Bayer AG, Leverkusen, F.R.G. The internal standard solution was nitrendipine (50 ng/ml) in toluene. All solutions were protected from light and stored at 4°C. Toluene (analytical-reagent grade) was from Merck (Darmstadt, F.R.G.).

Apparatus

A Varian 2100 gas chromatograph equipped with ^{63}Ni electron-capture detector (d.c. mode) was used. The GC column was glass (180 cm × 2 mm I.D.) filled with 3% OV-17 (Pierce, Rockford, IL, U.S.A.) on Gas Chrom Q, 80—100 mesh, conditioned for 24 h at 280°C with a nitrogen flow-rate of 25 ml/min. The operating conditions were: column temperature 255°C, injector temperature 255°C, detector temperature 290°C and carrier gas (nitrogen) flow-rate 25 ml/min. Under these conditions, the internal standard (nitrendipine) and nimodipine had retention times of 1.9 and 3.2 min, respectively.

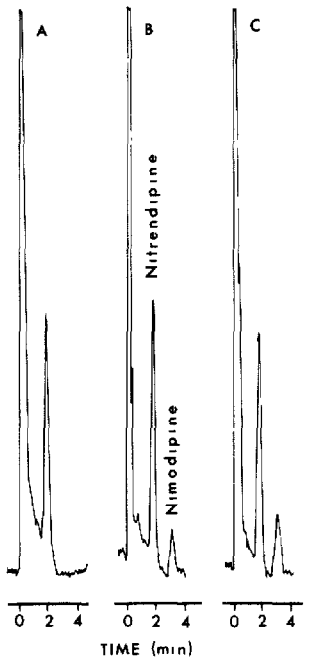
Procedure

The plasma sample (200 μl) was added to a microcentrifuge tube containing 100 μl of toluene with 50 ng/ml internal standard. The tube was whirly-mixed for 30 s and centrifuged for 2 min at 10 000 *g* (Beckman microfuge). Depending on the detector sensitivity, 1—5 μl of the organic phase were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Determination of nimodipine

Fig. 1 shows gas chromatograms of extracts of blank plasma (A), blank plasma spiked with 10 ng/ml nimodipine (B) and a patient plasma sample containing 15.2 ng/ml nimodipine (C). Although Krol et al. [9] found, depending on the GC conditions, that 5—60% of an injected amount of nimodipine is oxidized to the pyridine analogue in the gas chromatograph, only minimal degradation (< 5%) of both nimodipine and nitrendipine occur with our GC conditions. Our findings, which are not in full accordance with the findings of Krol et al. [9], might be the result of a lower injection temperature and of the injection of compounds directly into the column material. Standard



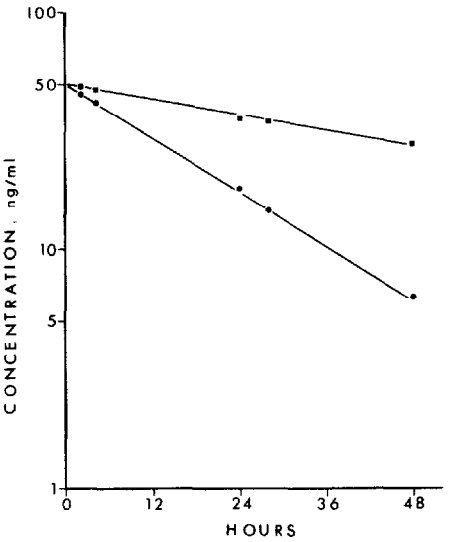


Fig. 1. Gas chromatograms of human plasma extracted with toluene containing the internal standard nitrendipine. (A) Plasma blank; (B) plasma spiked with 10 ng/ml nimodipine; (C) patient plasma containing 15.2 ng/ml nimodipine.

Fig. 2. Degradation of nimodipine in aqueous solution after exposure to daylight (▪) and UV radiation (•).

curves were linear up to 100 ng/ml. The limit of quantitation is ca. 0.5 ng/ml. Within-run precision was determined from six separate determinations of plasma samples containing 25 and 75 ng/ml nimodipine. The within-run coefficient of variation was found to be 3.3% for 25 ng/ml and 2.1% for 75 ng/ml nimodipine.

Effects of light

The acute clinical administration of nimodipine implies direct application of nimodipine on the cerebral vessels during operation using microscopical light of high intensity. Furthermore, intravenous administration via infusion sets implies a variable exposure to light. Previous studies in our laboratory have shown that the structurally close related dihydropyridine (nifedipine) is very sensitive to daylight ($t_{1/2}$ = 14 min) [8], and, therefore, had to be carefully protected from light. Nimodipine in aqueous solution was exposed to microscopical light and to daylight as well. Degradation under UV light (360 nm, 300 lux) was also demonstrated. It was found that nimodipine was resistant to microscopical light during the surgical procedure (ca. 30 min), while exposure to daylight and to UV light showed some degradation (Fig. 2). The degradation obeys first-order kinetics. Half-lives for nimodipine under the influence of daylight and UV light were calculated to be 56 and 16 h, respectively. Exposure to light for short periods, therefore, should not influence the concentration of nimodipine.

Loss of nimodipine due to absorption by PVC

Previous studies have shown that intravenous administration of nitroglycerine via PVC infusion sets (Steritex®, Mölnlycke, Sweden) resulted in a 40—80% loss of the drug, while administration via PVC-free sets (Accuset®, Imed, San Diego, CA, U.S.A.) did not affect the concentration of nitroglycerine delivered [10, 11].

Our data (Fig. 3) indicate that nimodipine, like nitroglycerine, is absorbed by PVC tubing. The concentration of nimodipine in the infusion solution decreases by ca. 20% during an experimental perfusion period of 5 h through normal PVC infusion sets. No loss of nimodipine is found when PVC-free tubes are used for the infusion. Previous studies [12] have demonstrated a correlation between clinical effects and plasma concentrations of dihydropyridines. It is therefore important to obtain a constant and sufficient delivery of nimodipine to the patient during the period of treatment. Our data show that this can be achieved when PVC-free tubes are used for the intravenous infusion.

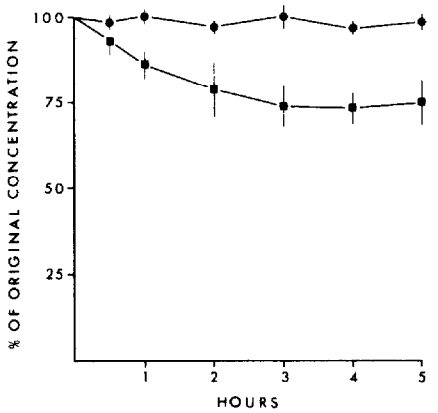


Fig. 3. Absorption of nimodipine by PVC-free (Accuset) (•) and PVC tubes (Steritex) (■).

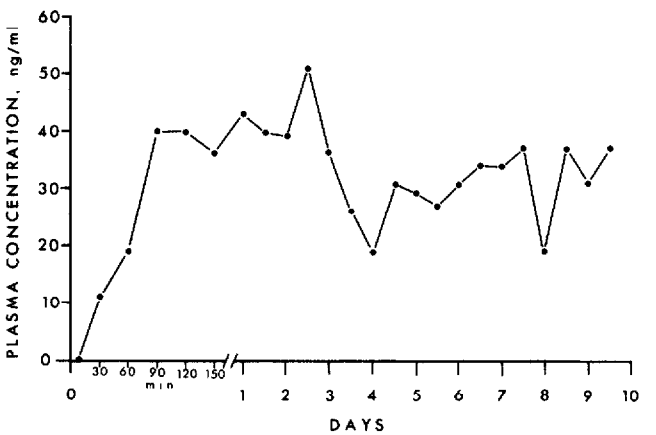


Fig. 4. Plasma concentrations of nimodipine during ten days of continuous intravenous infusion to a patient operated on for ruptured aneurysm.

Intravenous administration of nimodipine

Patients operated on for ruptured aneurysms were post-operatively treated with an intravenous infusion of nimodipine (1—2 mg/g). Heparinized blood samples were drawn 0, 10, 30, 60, 90, 120 and 150 min after the start of infusion and thereafter with an interval of 12 h for ten days. The blood samples were centrifuged and plasma was stored at −20°C until analysis was carried out. Fig. 4 shows the results of the determinations of plasma samples from one patient receiving 1 mg of nimodipine per hour. Steady-state plasma concentration of nimodipine was established within 1.5 h. Although some fluctuations appeared, presumably due to changes in the i.v. administration, the steady-state nimodipine concentration was held virtually constant throughout the period of administration. Further studies concerning the pharmacokinetics of nimodipine are in progress.

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