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The relevance of nitrendipine erythrocyte partitioning for the variability of its bioavailability parameters

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Abstract

The pharmacokinetic parameters of nitrendipine were determined in 40 healthy male volunteers and a very high degree of intersubject variability was observed (CV = 39–71%). Since the distribution of nitrendipine to erythrocytes could influence the overall pharmacokinetic variability the correlation between hematocrit and various pharmacokinetic parameters was analyzed, using linear regression. In vitro partitioning of nitrendipine to erythrocytes suspended in physiologic saline was studied over a range of hematocrit and drug concentration values. The correlations of in vivo pharmacokinetic parameters were linear with medium to high correlation coefficients (0.65–0.80). The positive correlation between the volume of distribution and hematocrit and negative between AUC, C_{max} and beta parameters indicate that nitrendipine enters and/or is bound to erythrocytes. The results of the in vitro erythrocyte partitioning experiments confirm this observations as the mean values of partition coefficient to erythrocytes was found to be 2.85 ± 0.17.

Keywords: Nitrendipine; Pharmacokinetics; Pharmacokinetic-hematocrit dependence; Erythrocyte partitioning

1. Introduction

Nitrendipine (3-ethyl-5methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)3,5-pyridine dicarboxylate) is a potent vasodilator with antihypertensive activity (Santiago and Lopez, 1990), whose effects are related to calcium channel inhibiting properties. The pharmacokinetic parameters of the drug reveals good absorption with first pass effect, very large volume of distribution and more than 95% binding on plasma protein (Kann et al., 1984; Goa and Sorkin, 1987). A very high degree of variability of pharmacokinetic parameters is observed, due to the differences in hepatic metabolism (Mikus and Eichelbaum, 1987) and plasma protein concentration (Raemsch et al., 1987).

The distribution of nitrendipine to erythrocytes should not be neglected as a contributing factor to the overall pharmacokinetic variability (Hinderling, 1997). At a given amount of drug in the blood, increased binding to erythrocytes could

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lead to its lower plasma levels. The purpose of this study was to assess the influence of hematocrit values, measured in healthy volunteers, on the pharmacokinetic parameters of nitrendipine and to relate the findings with erythrocyte partitioning/uptake/distribution data obtained for nitrendipine under in vitro conditions.

2. Methods

2.1. Clinical pharmacokinetic study

The study was designed as a comparative bioavailability study aimed to evaluate the relative bioavailability and bioequivalence of two nitrendipine 20 mg tablet formulations taken from the market in Slovenia (A and B).

The study was a single dose, blind, randomized, cross over design in forty normal, healthy male volunteers with the age from 18 to 30 years.

Every trial subject was asked to give consent to participate in the study by signing the informed consent statement. The study protocol was approved by the National Ethical Committee and the National Drug Regulatory Authority.

2.2. Pre-study examination

All subjects underwent a detailed medical examination in which all inclusion criteria planned in the trial protocol were assessed. Preclinical laboratory tests included complete haematological, biochemical screens including hematocrit, urinanalysis, blood pressure, ECG, HBs antigen, anti-HCV and anti HIV antibody testing.

Marginally increased or decreased values occurred in some subjects and were prior to the beginning of the study considered as clinically nonrelevant.

2.3. Dosage and blood sampling

Subjects received one 20 mg nitrendipine tablet with 100 ml of tap water during each study phase, according to the computer generated sequence with randomized attribution. Between the study sessions there was a 1-week wash out period. Sixteen blood samples were taken after each drug administration, centrifuged 10 min at 3000 rpm and the plasma was separated into polypropylene tubes. The plasma samples were stored at -18 ± 3°C until assayed. A suitably validated and sensitive GC-MS method was used for the analysis of nitrendipine in the plasma samples.

2.4. Nitrendipine measurement in the plasma

The plasma samples were investigated for their concentrations of nitrendipine by means of validated GC-MS method with negative chemical ionization.

Frozen plasma samples were thawed at ambient temperature. To 1 ml of plasma sample, the internal standard working solution and NaOH were added, the sample homogenized and extracted with n-pentane/ethylacetate mixture. After extraction the samples were centrifuged for 10 min at 13 000 rpm. Sample preparation was performed under yellow light. An aliquot of organic phase was transferred into a microvial and injected into the GC-MS instrument.

A GC-MS analysis was performed with a HP 5988 mass spectrometer with HP 5890 gas chromatograph and HP 7673 autosampler (Hewlett-Packard, Waldbronn, Germany). Separation was achieved using a J and W fused silica DB-1 capillary column (25 m $\times$ 0.32 mm ID, 0.25 mm film thickness). MS detection of nitrendipine and internal standard was carried out after negative chemical ionization with selected ion monitoring mode; for nitrendipine at m/z 360 and 346 for internal standard.

The linear concentration range was 0.1–20 ng/ml and the limit of quantification was 0.1 ng/ml. The intra-daily variations were 2.47, 3.33 and 8.82% for the concentrations of 19.8, 1.98 and 0.129 ng/ml.

2.5. Erythrocyte partitioning of nitrendipine in vitro

Known amounts of nitrendipine were added to Erlenmeyer flask containing a suspension of erythrocytes in physiologic saline to produce concentrations of 9.56, 9.08, 8.06, 7.65, 5.74 and 3.82

nmol/ml of nitrendipine and hematocrit values of 0.000, 0.026, 0.056, 0.105, 0.210 and 0.310. The samples were then incubated for 30 min at 37°C, centrifuged 10 min at 3000 rpm. The concentration of nitrendipine in the corresponding supernatant was measured with a GC-MS method described above.

2.6. Calculations

The comparative bioavailability pharmacokinetic parameters of nitrendipine were obtained from plasma concentrations by the SAS program (SAS/STAT Users Guide, 1990). The area under plasma concentration versus time ($0 \leq t \leq \infty$) curve was calculated by the trapezoidal rule and extrapolated to infinity:

$$\mathrm{AUC} = \mathrm{AUC}_{0-t} + \mathrm{AUC}_{t-\infty}$$

where t is the time of last sample.

$$\mathrm{AUC}_{0-t} = \frac{\sum(C_{i+1} + C_i)}{2(t_{i+1} - t_i)}.$$

where n is the number of sampling times, indices i and $i+1$ denote experimental data obtained at two consecutive sampling times and

$$\mathrm{AUC}_{t-\infty} = \frac{C^*}{\beta}$$

where C^* is the nitrendipine concentration at time t and β is the terminal plasma elimination rate constant, calculated from the linearly aligned points in the semilogarithmic plot of nitrendipine concentrations versus time by least squares linear regression.

Volume of distribution was obtained by the equation

$$V_{ss} = D \times F \times \frac{\mathrm{AUMC}}{\mathrm{AUC}^2}$$

where D is dose and AUMC is the area under the first moment curve of nitrendipine concentrations versus time and F is the fraction of the dose reaching systemic circulation. The bioequivalence of the two formulations was assessed according to the International guidelines (Jusko, 1986).

The extent and the shape of correlation between pharmacokinetic parameters and the hematocrit were determined using linear regression and the Pearson's coefficient of correlation. The tool used for the statistical analysis SPSS (SPSS, 1997) anol SAS(SAS/STAT Users Guide 1990).

The extent of nitrendipine erythrocyte partitioning was expressed as the partition coefficient (P). It was obtained by measuring the respective concentrations of the drug in the supernatant (C_s), the hematocrit (H_c) and calculating the respective concentrations of the intracellular nitrendipine assuming mass-balance conditions. The following equations were used in the calculations of P and C_e:

$$P = \frac{C_e}{C_s} \tag{1}$$

where, P is the partition coefficient, defined as the ratio of nitrendipine concentration in the erythrocytes to its concentration in the supernatant under steady-state conditions. The erythrocyte concentrations of the drug were calculated to the following equation:

$$C_e = \frac{C_0 - C_s(1 - H_c)}{H_c} \tag{2}$$

where, C_0 was the initial concentration of nitrendipine in suspension, C_s the concentration of nitrendipine in supernatant and H_c the hematocrit.

Fraction of the drug distributed to erythrocytes (F_e) was calculated as the fraction of the amount of drug in the erythrocytes versus the amount of drug in the suspension

$$F_e = C_e \frac{H_c}{C_0} \tag{3}$$

3. Results

Pharmacokinetic profiles for both preparations of nitrendipine (means and standard deviations) obtained in the clinical pharmacokinetic study are shown in Fig. 1.

Correlation between hematocrit and pharmacokinetic parameters was analyzed.

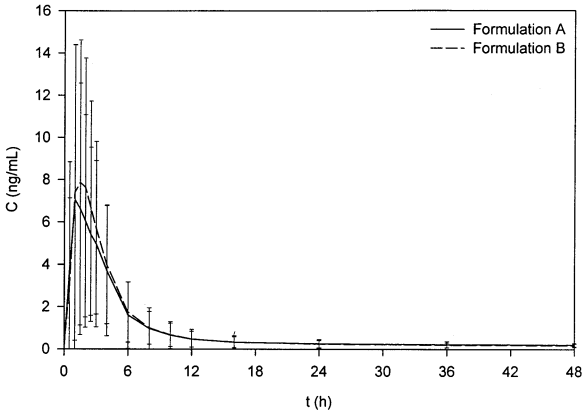

Fig. 1. Pharmacokinetic profiles of nitrendipine for studied preparations (mean and S.D., $n = 40$) following oral application of 20 mg dose.

The mean value of hematocrit of 40 volunteers was 0.44 ± 0.02 and the range from 0.385 to 0.497 showed that all the volunteers had normal values.

The pharmacokinetic parameters were calculated from the plasma concentrations of nitrendipine of same volunteers after single dose of 20 mg of nitrendipine. The mean values of main pharmacokinetic parameters with standard deviations and coefficients of variation are shown in Table 1.

The correlation between hematocrit and pharmacokinetic parameters was made according to a linear regression program and is presented in Fig. 2.

Table 1
Main pharmacokinetic parameters of nitrendipine (mean ± S.D.) with coefficients of variation (CV)

Parameter	Value ± S.D.	CV (%)
Area under curve (AUC)	33.57 ± 13.22 ng/ml per h	39
Maximal concentration (C_{max})	8.04 ± 4.49 ng/ml	53
Elimination rate constant (β)	0.096 ± 0.068/h	71
Bioavailability-normalized volume of distribution (V_{ss}/F)	5347 ± 2783 l	52

3.1. Nitrendipine partitioning to erythrocytes

From the in vitro partitioning data the partition coefficient P, relating the intra- to extra-cellular drug concentration could be derived. The characteristics of nitrendipine association with erythrocytes are summarized in Table 2.

The partitioning of nitrendipine with erythrocytes was determined at different hematocrit values in the experimental system and at different concentrations of nitrendipine. The mean observed erythrocyte/supernatant partition coefficient P was 2.85 ± 0.17. This indicated that nitrendipine was not only distributed but also bound to cellular elements within and/or on the erythrocytes. Surprisingly, the binding appeared to be nonsaturable in our experimental conditions over the range of nitrendipine concentrations.

4. Discussion

Calcium channel antagonists of the dihydropyridine type show marked inter- and intra-individual differences in their pharmacokinetic response which in the literature are most frequently attributed to the differences in the rate and extent of their biotransformation and drug protein binding. Due to the fact that many of these drugs also distribute to a large extent to erythrocytes (Urien et al., 1985; Pinquier et al., 1988), it was anticipated in this study that erythrocyte binding would influence pharmacokinetic response. In the present pharmacokinetic study using nitrendipine, large intersubject variations in plasma concentrations were found. As assay methods determine only total plasma concentrations (sum of free and bound plasma concentrations), their variability is influenced also by their partition to erythrocytes. Using linear regression, we assessed, therefore, the dependence/sensitivity of pharmacokinetic parameters on the hematologic parameters, i.e. the hematocrit. Clinical pharmacokinetic study, in which 20 mg single dose nitrendipine was administered orally to 40 volunteers in the form of two bioequivalent formulations was used as the experimental base. The intersubject coefficient of variation for all pharmacokinetic parameters in the

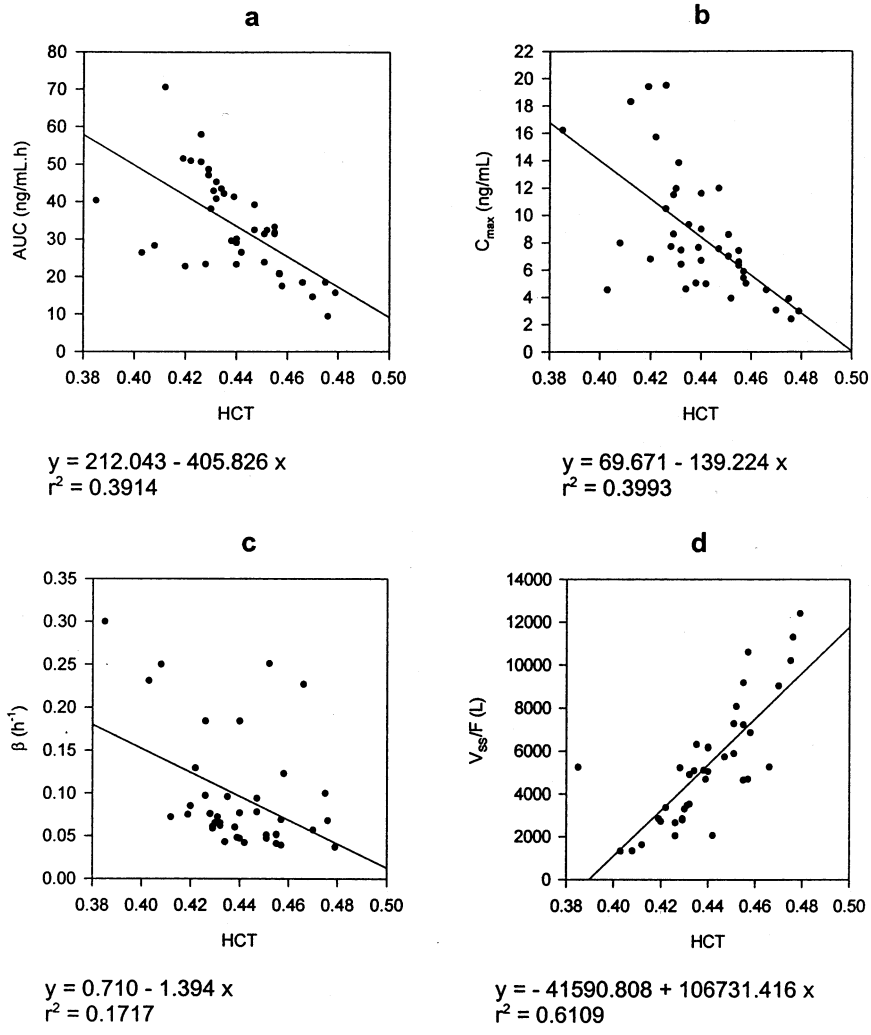

Fig. 2. Linear regression between hematocrit and pharmacokinetics of nitrendipine: (a) area under the concentration-time curve; (b) maximum plasma concentration; (c) elimination rate constant; (d) volume of distribution.

study was greater than 39%, in the case of the bioavailability-normalized volume of distribution the range of values spanned over 1:10 ratio. The dependence of pharmacokinetic parameters from the measured hematocrit values in participating volunteers showed linear dependence with intermediate to high correlation coefficients (0.65–0.80). The positive correlation between the volume of distribution and hematocrit and negative correlation between AUC, C_{max} and beta parameters indicate that nitrendipine enters and/or is bound to erythrocytes. An increased hematocrit would thus provide more distribution space for erythrocyte uptake thus lowering the amount of drug in plasma and likely also the plasma concentration. In turn, lowered plasma concentration would lead to increased values of the volume of distribution producing the positive correlation.

In order to confirm this observation, in vitro erythrocyte partitioning experiments were performed, in which erythrocyte suspension in physiologic saline with a predetermined hematocrit was taken as experimental system. It was found that nitrendipine distributes to erythrocytes to a large

Table 2
The erythrocyte partitioning of nitrendipine in suspension of erythrocytes[a]

N (μg)	H_c	C_s (nmol/ml)	F_e	C_e (nmol/ml)	P
40.0	0.000	9.56	0.000	0.00	0.00
38.0	0.026	8.70	0.067	23.40	2.69
36.0	0.052	7.88	0.216	22.00	2.79
32.0	0.105	6.26	0.269	19.59	3.12
24.0	0.210	4.11	0.433	11.85	2.88
16.0	0.314	2.48	0.554	6.74	2.71

[a] N, nitrendipine added to system; H_c, hematocrit; C_s, concentration of nitrendipine in the supernatant; F_e, fraction amount of drug in erythrocytes; C_e, erythrocyte concentration of nitrendipine; P, partition coefficient.

extent, whereby, both partitioning and binding to the components of the cytosol and/or the cellular membrane can be considered. The concentration of nitrendipine in the supernatant was much lower compared with its expected value in the case, if a simple equilibration between the two compartments would be occurring. Therapeutic concentration range was chosen for the in vitro partitioning experiments in order to obtain comparative data with those from the in vivo study. Consequently, the concentrations of the drug in the suspension were relatively low and no saturation of binding was taking place under the equilibrium conditions. The value of the partition coefficient namely P remained relatively constant throughout the experimental ranges of nitrenidipine concentrations and hematocrit. It was shown that the erythrocytes are an important distribution compartment for nitrendipine and likely for other calcium antagonists. The differences in hematocrit in the physiologic range of the normal volunteers study could influence to a marked extent the variability of the key pharmacokinetic parameters such as AUC, C_{max} and beta. The volunteers should thus be carefully chosen to participate in the clinical pharmacokinetic studies and, therefore, attention should be given to the measured values of hematocrit in the volunteers entering studies with this type of drugs. Even more so, differences in partitioning could play a role in the patients with unexpected pharmacokinetic/pharmacodinamic response to nitrendipine.

References

Goa, K.L., Sorkin, E.M., 1987. Nitrendipine: a review of its pharmacodinamic and pharmacokinetic properties and therapeutic efficacy in the treatment of hypertension. Drugs 33, 123–135.

Hinderling, P.H., 1997. Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. Pharmacol. Rev. 49, 279–292.

Jusko, V.J., 1986. Guidelines for collection and analysis of pharmacokinetic data. In: Evans, V.E., et al. (Eds.), Applied Pharmacokinetics, second ed. Applied Therapeutics, Inc, Spokane WA.

Kann, J., Krol, G.J., Raemsch, K.D., 1984. Bioequivalence and metabolism of nitrendipine administered orally to healthy volunteers. J. Cadriovasc. Pharmacol. 6, S968–S973.

Mikus, G., Eichelbaum, M., 1987. Pharmacokinetics, bioavailability, metabolism and hemodynamic effect of the calcium channel antagonist, nitrendipine. J. Cardiol. Pharmacol. 9, S140–S141.

Pinquier, J.L., Urien, S., Lemaire, M., Chaumet-Riffaud, P., Tillement, J.P., 1988. Comparative binding of two closely related dihydropyridines (isradipine and darodipine) to serum proteins and erythrocytes. Pharmacology 36, 305–312.

Raemsch, K.D., Graete, K.H., Scherling, D., 1987. Pharmacokinetics and metabolism of calcium blocking agents — nitrendipine, nifedipine and nimodipine after i.v. and po administration. Br. J. Clin. Pharmacol. 24, 561–569.

Santiago, T.M., Lopez, L.M., 1990. Nitrendipine: a new dihydropyridine calcium channel antagonist for the treatment of hypertension. Ann. Pharmacother. 24, 167–175.

SAS/STAT Users Guide, Version 6, fourth ed., Vol. 2, SAS Institute Inc., Cary, NC 1990, 1195–1210.

SPSS Advanced Statistics 7.5, SPSS Inc., Chicago III. 1997, 89–98.

Urien, S., Albengres, E., Compte, A., Kiechel, J.R., Tillement, J.P., 1985. Plasma protein binding and erythrocyte partitioning of nicardipine in vitro. J. Cardiovasc. Pharmacol. 7, 891–989.