implement in long period forecasting of grain. *Exploration of Nature* 1984; 3(3) : 37
2 Hammer R, Berrie CP, Birdsall NJM, Burgen ASV, Hulme EC. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* 1980; **283** : 90
3 Luduena FP, Lands AM. An investigation of the pharmacological actions of three antispasmodic compounds and their corresponding metho-salts. *J Pharmacol Exp Ther* 1954; **110** : 282
4 陆守曾. 曲线回归. 见: 郭祖超, 主编: 医用数理统计方法. 北京: 人民卫生出版社, 1988: 573-630
5 邓聚龙. 灰色系统基本方法. 武汉: 华中工学院出版社, 1987: 59-60
6 Ostle B. *Statistics in research*. 2nd ed. Ames: Iowa State Univ Press, 1963 : 222-49
7 Garmine AA, Brogden RN. Pirenzepine: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in peptic ulcer disease and other allied diseases. *Drug* 1985; *30*: 85

预测药物远期效应的灰色动态模型及其在哌仑西平的应用

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提要 为定量描述药物效应的动态变化和预测药物远期效应，本文建立了灰色动态模型并应用于预测给狗服用哌仑西平 104 mg / (kg · d)对瞳孔及外周白细胞的远期影响. 基于此模型，拟合给药前及给药 16 wk 期间瞳孔直径. 中性粒细胞及淋巴细胞百分比的实验数据，得到预测该药远期效应的方程式. 由此方程式计算的给药后 20 和 24 wk 的预测值与观察值之间的相对误差较小，提示预测是可行的.

关键词 药效学; 哌仑西平; 瞳孔; 白细胞; 数学计算

中国药理学报 *Acta Pharmacologica Sinica* 1990 Nov; 11 (6) : 484-487

Pharmacokinetics of *m*-nisoldipine in rabbits and rats

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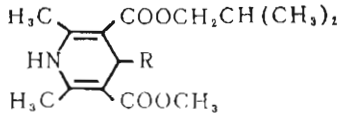
ABSTRACT A reverse phase HPLC method was devised for determination of *m*-Nis in plasma. A mobile phase of methanol- KH_2PO_4 with a flow rate of 1 ml / min was used. Diazepam was used as the internal standard. A two-compartment model featured the pharmacokinetic process of *m*-Nis after its iv injection to rats (30 μg / kg) and rabbits (50 μg / kg). The pharmacokinetic parameters were : $T_{\frac{1}{2}\alpha}$ = 4.3 min, $T_{\frac{1}{2}\beta}$ = 63.6 min, V_d = 0.805 L / kg, Cl = 9 ml / (min · kg) in rats; $T_{\frac{1}{2}\alpha}$ = 5.0 min, $T_{\frac{1}{2}\beta}$ = 78.3 min, V_d = 1.191 L / kg, Cl = 11 ml / (min · kg) in rabbits. The pharmacokinetics for *m*-Nis after ig 200 μg / kg to rats described one-compartment model with parameters: $T_{\frac{1}{2}}$ = 84.8 min, T_{max} = 31.2 min, C_{max} = 49.97 μg / L, V_d = 0.792 L / kg and Cl = 25 ml / (min · kg).

KEY WORDS *m*-nisoldipine; pharmacokinetics; high pressure liquid chromatography

Received 1989 Jul 6 Accepted 1990 Aug 23

m-Nisoldipine (*m*-Nis), a new isomer of nisoldipine (Nis), was first developed in Department of Organic Chemistry, Hebei Medical College. *m*-Nis is more stable than Nis to light[1,2]. It shows active effects on the cardiovasculature and hemodynamics[1,3-5] and is a perspective agent in cardiovascular therapeutics. This paper deals with HPLC determination of *m*-Nis and its pharmacokinetics in rats and rabbits.



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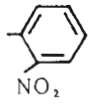

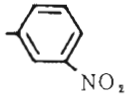

Nisoldipine *m*-Nisoldipine

MATERIALS AND METHODS

Chemicals and Reagents *m*–Nis, isobutyl methyl 1,4–dihydro–2,6–dimethyl–4–(3–nitrophenyl)–3,5–pyridine dicarboxalate, was obtained from Department of Organic Chemistry, Hebei Medical Colldge. The tertiary butyl methyl ether (TBME) of chromatographic grade was used and all other chemicals and reagents were of AR. Water was distilled in glass and filtered with a 0.45 μm Millipore.

Solutions Stock solutions of *m*–Nis and diazepam (Dia, the internal standard) 1 mg / ml were prepared with methanol, separately. The standard solution of *m*–Nis (0.15–9 μg / ml) containing Dia 0.3 μg / ml was made by diluting the stock solutions with mobile phase before use.

Apparatus The HPLC system consisted of a model 510–A solvent delivery system, a model U6K universal injector and a model 440 uv deterctor (Waters Assoc).

Chromatographic conditions Samples were analysed at 22 ± 1 ℃ on a reverse phase column of 30 cm × 3.9 mm id (μ–Bondapak C_{18}, Waters Assoc). Mobile phase consisted of methanol–KH_2PO_4 of 15 mmol / L (vol : vol). The flow rate was 1 ml / min (16 MPa). The sensitivity of uv detector was set at 0.01 absorbance unit of full scale (AUFS). The chromatograms were traced on an automatic equilibrium recorder (model XWT–264 Dahua Instrument Factory, Shanghai).

Animal Experiments Six rabbits, ♂ weighing 2.85 ± SD 0.15 kg and after over–nigh fasting, were given a bolus dose of *m*–Nis 50 μg / kg in the ear vein. Blood samples were collected at 0, 2, 5, 10, 15, 20, 35, 60, 120 and 180 min. Forty–five Wistar rats, ♂ & ♀ weighing 300 ± 40 g, were randomly divided into 9 groups. The blood samples were obtained from heart at 0, 2, 5, 10, 15, 35, 60, 90 and 180 min respectively after a bolus injection of *m*–Nis 30 μg / kg in the tail vein. For study of ig dosing, *m*–Nis 200 μg / kg was given to rats and blood samples were drawn from heart at 0, 10, 20, 35, 60, 180, 300, 480 and 600 min respectively. The plasma was stored in refrigerator untill use.

Sample Preparation Plasma 1 ml was mixed with Dia 18 ng; and NH_4OH 200 μl were dripped in gradually with shaking. Then TBME 5 ml was added. The mixture was centrifuged at 1600 × g for 10 min. The supernatant layer was evaporated to dryness with a vacuum aspirator at 50 ℃. The residue was dissolved with 60 μl of mobile phase and aliquot of 20 μl was injected the liquid chromatography.

Quatification The blank plasma was spiked with *m*–Nis 3–180 ng / ml. Calibration curve was obtained by plotting the peak height ratio of *m*–Nis to Dia *VS* the amount of *m*–Nis added.

Pharmacokinetic Analysis Data of plasma concentration–time were fitted and the pharmacokinetic parameters were processed on an Apple–II microcomputer[6]. The mathematical model yielded the lowest Akaike Information Criterion (AIC) value[7]. The bioavailibility (F) was calculated according to:

$$F = [Div \cdot (T_{\frac{1}{2}\beta})_{iv} \cdot AUC_{oral}] / [D_{oral} \cdot (T_{\frac{1}{2}\beta})_{oral} \cdot AUC_{iv}]^{(8)}$$

RESULTS AND DISCUSSION

Chromatographic Behaviors The uv absorption maximum of *m*–Nis was at 234.8 nm, but the absorbance at 254 nm was used for detection. It provided a clear chromatogram for blank plasma, because some endogenous plasma substances reduced their sensitivity of uv absorption at 254 nm.

A good separation of standard *m*–Nis and Dia was obtained by using methanol and water (4:1, vol:vol) as mobile phase, but the peaks of endogenous plasma substances appeared in chromatogram. However, an acidic mobile phase with methanol and buffer (15 mmol / L)

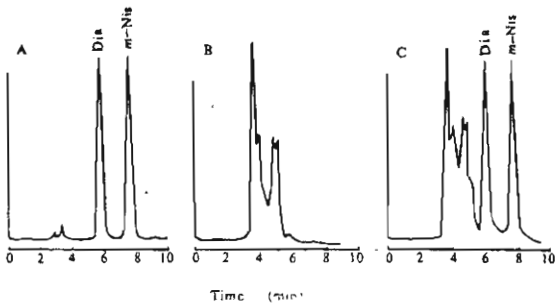

Fig 1. Liquid chromatograms: (A) diazepam 6 ng & *m*–Nis 16 ng standards; (B) 1 ml blank plasma; (C) 1 ml blank plasma with diazepam 18 ng & *m*–Nis 48 ng. AUFS 0.01.

improved the chromatogram of blank plasma. The typical chromatogram (Fig 1) showed the retention times of Dia and *m*–Nis were 5.8 and 7.5 min respectively.

Analytical Variables Result revealed an excellent linearity of calibration curve $r = 0.9997$ (Fig 2).

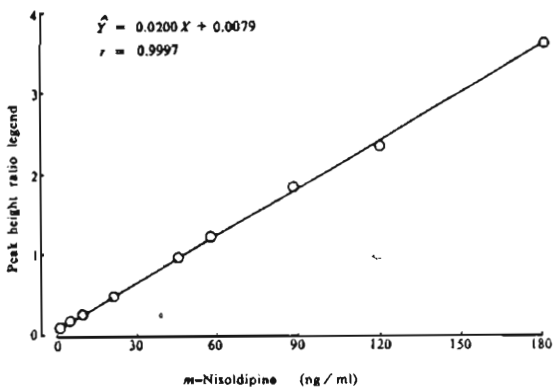

Fig 2. Calibration curve of *m*–Nis in plasma.

The recovery of *m*–Nis form plasma averaged 99.95± 1.92 %. The coefficients of variation within day and between days were <6% at all concentrations. The analytical variables identified significant reliability and reproducibility of the method we developed.

Pharmacokinetics The concentration–time curve of *m*–Nis in plasma was shown in Fig 3.

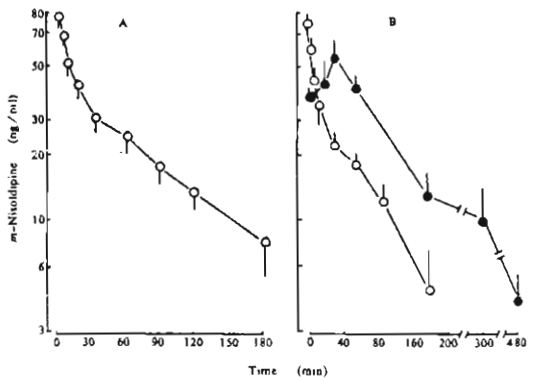

Fig 3. Concentration of *m*–Nis in plasma after iv 50 μg / kg to rabbits, iv 30 μg / kg to 6 rats (O) and ig 200 μg / kg to rats (●).

The combination of ideal fitting curve with AIC and *r* values were shown in Tab 1.

Tab 1. Estimation of compartment model for *m*–Nis (*r* = correlation coefficient)

			One–compartment		Two–compartment	
Animal	(*n*)	Route	AIC	*r*	AIC	*r*
Rabbit	(6)	iv	72.99	0.899	48.73	0.994
Rat	(45)	iv	52.89	0.947	11.94	0.999
Rat	(45)	ig	45.06	0.992	51.39	0.988

The pharmacokinetic processes of *m*–Nis following iv dosing in rabbits and rats corresponded much more with two–compartment model. However, Tab 2 shows that the distribution phase ($T_{\frac{1}{2}\alpha} = 4.3$ min) is faster than the absorption phase ($T_{max} = 31.2$ min). Moreover, no clear distribution phase can be distiguished after ig dosing of *m*–Nis to rats (Fig 3B), so the pharmacokinetic process of *m*–Nis following ig dosing feartures one–compartment model.

Tab 2 summarizes the pharmacokinetic parameters of *m*–Nis. It describes a rapid distribution and elimination of *m*–Nis in animals. The fact that K_{12} was greater than K_{21} indicates that *m*–Nis quickly distributed to the peripheral compartment. The volume of apparent distribution is twice the volume of center distribution (Tab 2). This finding in–

Tab 2. Pharmacokinetics of *m*-Nis in 6 rabbits and 45 rats.

Parameters	Rabbits	Rats	
	iv 50	iv 30	ig 200 μg / kg
$\alpha(min^{-1})$	0.161	0.160	
$\beta(min^{-1})$	0.009	0.011	0.008
$K_{12}(min^{-1})$	0.081	0.081	
$K_{21}(min^{-1})$	0.068	0.062	
$K_{10}(min^{-1})$	0.021	0.028	
$T_{\frac{1}{2}\alpha}$(min)	5.0	4.3	
$T_{\frac{1}{2}\beta}$(min)	78.3	63.6	84.8
$K_a(min^{-1})$			0.083
$T_{max}(min^{-1})$			31.2
$C_{max}(\mu g / L)$			49.97
V_d(L / kg)	1.191	0.805	0.792
V_c(L / kg)	0.504	0.313	
Cl (ml · kg^{-1} · min^{-1})	11.0	9.0	25.0
AUC(μg · min / L)	4789.75	3418.80	7719.11

tegrated with the high extent of *m*-Nis binding to plasma protein in our another experiments permits us to consider the extensive tissue distribution of the drug. Our result is similar to those of other authors[9-11].

The quick absorption with peak time of 0.5 h and $T_{\frac{1}{2}\beta}$ of 1.41 h was similar to that of Nis[12] which $T_{\frac{1}{2}}$ was markedly prolonged with increasing dose.

The *m*-Nis shows a low bioavailability of 25.4 % in our study. The bioavailability of other 1,4-dihydropyridines is not high either and the powerful first-pass hepatic metabolism is considered to contribute to the low bioavailability[9-12].

REFERENCES

1 Ren LM, Li YS, FU SX, Jin CJ. Cardiovascular action of *m*-nisoldipine in anesthetized rabitts and guinea pigs. *Acta Pharmacol Sin* 1988; **9** : 426

2 Yan TR, Wu YW, Zhao JQ, Nei H, Yuan FY. Studies on photostability of new calcium channel blocking agent nisoldipine. *Acta Acad Med Hebei* 1987; **8**: 265

3 Fu SX, Li YS, Jin CJ, Ren LM. Effects of *m*-nisoldipine and nisoldipine on hemodynamics in anesthetized dogs. *Acta Pharmacol Sin* 1988; **9** : 43

4 Li YL, Fu SX, Li YS. Prophylactic effects of *m*-nisoldipine and nisoldipine on reperfusion arrhythmia in hearts of rats. *Ibid* 1988; **19**: 542

5 Zhang HL, Fu SX, Li YS. Protective effects of *m*-nisodipine and nisodipine on myocardial damage in working rabbit hearts after ischemia-reperfusion. *Ibid* 1989; **10** : 49

6 Peng B, Zhao XL. A computer program for calculating pharmacokinetic constants following iv administration of drug. *Acta Zhongshan Med Univ* 1987; **8** : 55

7 Yamaoka K, Nakagawa T, Uno T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J Pharmacokinet Biopharm* 1978; **6** : 165

8 Gibaldi M, Perrier D. eds. *Pharmacokinetics*. 2nd ed. NY: Marcel Dekker, 1982 : 171 (Swarbrick J, ed. *Drug and the pharmaceutical sciences*; vol 15)

9 Sorkin EM, Clissold SP, Brogden RN. Nifedipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischemic heart disease, hypertension and related cardiovascular disorders. *Drugs* 1985; **30** : 182

10 Sorkin EM, Clissold SP. Nicardipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in the treatment of angina pectoris, hypertension and related cardiovascular disorders. *Ibid* 1987; **33** : 296

11 Goa KL, Sorkin EM. Nitrendipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the treatment of hypertension. *Ibid* 1987; **33** : 123

12 Takata Y, Kato H. Comparative study on acute antihypertensive effects and pharmacokinetics of nisodipine, nifedipine, nimodipine and nicardipine administered orally to conscious renal hypertensive dogs. *Arzneimittelforschung* 1986; **36** : 1464

间尼索地平在兔和大鼠体内的药物动力学

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提要 本文建立了一种测定血浆间尼索地平(*m*-nisoldipine, *m*-Nis)浓度的反相 HPLC 法. 采用甲醇-KH_2PO_4 为流动相, 流速 1 ml / min. 以地西泮为内标. iv 给药, 家兔 50 μg / kg, 大鼠 30 μg / kg, *m*-Nis 的药动学过程呈二室模型. 参数为: 大鼠 $T_{\frac{1}{2}\alpha}$ = 4.3 min, $T_{\frac{1}{2}\beta}$ = 63.6 min, V_d = 0.805 L / kg. Cl = 9 ml / (min · kg); 家兔 $T_{\frac{1}{2}\alpha}$ = 5.0 min, $T_{\frac{1}{2}\beta}$ = 78.3 min, V_d = 1.19 L / kg, Cl = 11 ml / (min · kg). 大鼠 ig 200 μg / kg, *m*-Nis 药动学过程呈一室模型. 其参数: $T_{\frac{1}{2}}$ = 84.8 min T_{max} = 31.2 min, C_{max} = 49.97 μg / L, V_d = 0.792 L / kg, Cl = 25 ml / (min · kg).

关键词 间尼索地平; 药物动力学; 高压液相色谱法