A Modified Product Inhibition Model Describes the Nonlinear Pharmacokinetics of Nicorandil in Rats[1]

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Nicorandil, a vasodilator which acts through both cyclic GMP accumulation and K^+ channel opening, has been used in the treatment of various cardiovascular diseases. We have examined the pharmacokinetics of nicorandil in the rat as a function of dose, as both i.v. boluses (9 doses, 0.75–12 mg, n = 1-4 per dose), and as a 5-hr infusion followed by a 5-hr washout (6 doses, 10-500 μg/kg/min, n = 3 per dose). Plasma nicorandil concentrations were determined by HPLC. Nicorandil plasma concentrations increased disproportionately with dose, but nicorandil elimination obeyed apparent monoexponential kinetics, and the apparent half-life ($t_{1/2}$) increased with dose. In addition, the approach to apparent steady-state during the infusion phase was not overtly sensitive to the drastic changes in $t_{1/2}$ observed. Pharmacokinetic modelling with several nonlinear models, viz: Michaelis-Menten with parallel first-order, cosubstrate depletion and competitive product inhibition, were carried out. Addition of the sulfhydryl donor, *N*-acetyl-L-cysteine, did not change the pharmacokinetics of nicorandil, providing experimental indication that a cosubstrate depletion model might not be applicable. To describe the unique pharmacokinetics, a modified product inhibition model was developed. This new model includes the classic competitive product inhibition equation, describing both parent and product kinetics, and it incorporates, in addition, separate first-order elimination rate constants for both nicorandil and the inhibiting metabolite. Experimental evidence showed that *N*-(2-hydroxyethyl) nicotinamide, the major metabolite of nicorandil in rats, and nicotinamide (niacinamide) itself, indeed inhibited nicorandil elimination.

KEY WORDS: nicorandil; product inhibition; dose-dependent pharmacokinetics; computer modelling.

INTRODUCTION

Organic nitrates such as nitroglycerin and isosorbide dinitrate are commonly used in the management of a variety of cardiovascular diseases, including angina pectoris (1) and congestive heart failure (2). The pharmacologically important functional group in these compounds is the organic nitro-ester, which is metabolically converted to nitric oxide (3), leading to an elevation of intracellular cyclic GMP and vasodilation. Nicorandil, (*N*-(2-hydroxyethyl) nicotinamide nitrate ester), a relatively new organic nitrate, in part exerts its action through this pathway, but it possesses an additional mechanism of action as that of a potassium channel activator. The potassium channel activity of nicorandil is apparently associated with its pyridyl moiety (4). It has been suggested that this dual mechanism of action for nicorandil may confer an advantage in that it may not cause the characteristic hemodynamic tolerance which plagues current nitroester therapy (5,6), although a recent study by us (using an experimental heart failure rat model) suggested that this theoretical advantage may not be achievable (7).

Few pharmacokinetic studies have been reported on nicorandil. In a study in healthy human subjects (8), nicorandil did not appear to exhibit pharmacokinetic nonlinearity at four oral doses between 5 and 40 mg, while in another study in congestive heart failure patients (9), oral doses ranging from 10 to 60 mg resulted in substantial pharmacokinetic nonlinearity in the area under the plasma concentration vs. time curve (AUC), and in the maximum observed plasma concentration (C_{max}). Studies in the rat by Sakai *et al.* (10) also suggested nonstationarity in nicorandil pharmacokinetics following several nicorandil intraduodenal doses, but detailed pharmacokinetic analyses were not conducted. Tice *et al.* (9) observed a disproportionate increase in nicorandil AUC with oral dose, and suggested that increased bioavailability might occur at high doses. It is not known whether systemic disposition of nicorandil after intravenous doses may also exhibit nonlinearity.

In a preliminary study using two intravenous doses of nicorandil in the rat (11), we observed that the elimination of the parent compound was mono-exponential in nature, but the elimination $t_{1/2}$ was dose-dependent; increasing the intravenous dose by 16-fold (0.75 to 12 mg) brought about a 3.6 fold increase in the $t_{1/2}$ (0.7 to 2.6 hr). In this experiment, the elimination phase from the higher dose was monitored for about 18 hrs and showed that the elimination $t_{1/2}$'s from the two doses were different even over the same concentration ranges. Thus, the nonlinear pharmacokinetics could not be readily ascribed by simple Michaelis-Menten behavior. In this study, early results indicated that dose-dependent changes in drug elimination were again observed, when nicorandil was infused intravenously, but the time taken to approach apparent steady-state did not appear to depend on the dose (data in this report). These interesting preliminary results led us to conduct this study to document these phenomena, and to analyze the pharmacokinetic features of nicorandil through various nonlinear pharmacokinetic models. We have also attempted to determine if the major metabolite of nicorandil and also a structurally similar compound, niacinamide, might have interfered with the pharmacokinetics of nicorandil.

MATERIALS AND METHODS

Materials

Methanol was of HPLC grade and ethyl acetate was of analytical grade (J.T. Baker, Phillipsburg, NJ). *N*-acetyl-L-cysteine, *N*-acetylserine and niacinamide were obtained from Sigma Chemical Co. (St. Louis, MO). Nicorandil, *N*-(3-hydroxypropyl) nicotinamide nitrate ester, and *N*-(2-hydroxyethyl) nicotinamide were gifts from Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

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I.V. Bolus Study

Male Sprague-Dawley rats (322 ± 6 g) were implanted with a jugular vein cannula under light ether anesthesia one day prior to the experiment. Drug solutions were prepared daily. Bolus doses of 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 6.0, 10.0 or 12.0 mg (n = 1-4 per dose) were administered as a short infusion (2.3 min) through the jugular vein cannula. Following bolus dosing, the cannula was flushed with physiologic saline. Aliquots of 0.5 ml of jugular blood were sampled prior to bolus dosing (for blank plasma) and periodically after drug administration.

I.V. Infusion Study

Male Sprague-Dawley rats (317 ± 5 g) were implanted with femoral and jugular vein cannulas under light ether anesthesia one day prior to drug dosing. On the study day, the dosing solutions were prepared at appropriate concentrations so that the same total volume (5.82 ml) was infused into each animal, irrespective of dose. Between 8:00-8:30 a.m., 5.0 hr infusion at 10, 25, 50, 100, 200 or 500 μg/kg/min (n = 3 per infusion rate) was initiated through the femoral cannula at 19.4 μl/min with a Harvard infusion pump (South Natik, MA). Blood samples of 0.5 ml were taken from the jugular cannula at -0.5, 2.5, 4.0, 5.0, 5.5, 6.0, 6.5, 7.5, 8.5 and 10.0 hr. Blood was immediately centrifuged and the plasma was stored at -20°C in a microcentrifuge tube until HPLC analysis. Red blood cells were resuspended in physiologic saline and infused back to the same rat after the next sample was drawn. Animals were conscious, unrestrained and allowed free access to food and water throughout the study.

HPLC Analysis

Nicorandil concentrations in plasma were analyzed by an adapted HPLC assay (10,11). Briefly, an aliquot of plasma (200 μl) was mixed with 10 μl of *N*-(3-hydroxypropyl) nicotinamide nitrate ester (internal standard). The mixture was extracted with 5 volumes of ethyl acetate twice and solvent layers were combined and evaporated under N_2. The residue was immediately reconstituted with 200 μl of double distilled water and 30 μl was injected on a Waters C_{18} μBondapak 3.9 mm (ID) × 30 cm column. The mobile phase consisted of 45% methanol/double distilled water at a flow rate of 1.0 ml/min. Detection was carried out with a Hewlett-Packard 1040M photo-diode array detector at 254 nm. The retention times for *N*-(2-hydroxyethyl) nicotinamide (the primary metabolite), nicorandil and *N*-(3-hydroxypropyl) nicotinamide nitrate ester (the internal standard) were 3.5, 5.4 and 8.2 min, respectively. Nicorandil recovery from spiked plasma was found to be greater than 95% when compared to aqueous standards which were used daily for calibration. The lowest detectable limit for aqueous standards was about 8.7 ng on column, the inter- and intra-day coefficient of variation for the assay at nicorandil concentrations between 0.1 and 125 μg/ml were less than 5%.

Cosubstrate Depletion Study

Male Sprague-Dawley rats were implanted with jugular vein and femoral vein cannulas under light ether anesthesia the day prior to the experiment. Solutions of nicorandil, *N*-acetyl-L-cysteine (a sulfhydryl donor, 6.47 mg/kg/min) and *N*-acetylserine, (the hydroxy analog of *N*-acetyl-L-cysteine, 6.47 mg/kg/min) were prepared fresh daily. Nicorandil (200 μg/kg/min) and *N*-acetyl-L-cysteine (6.47 mg/kg/min) were coinfused for 3 hr through the femoral vein cannula. At 3 hr, the coinfusion solution was changed to nicorandil and *N*-acetylserine at the same respective doses, and this mixture was infused for an additional 3 hr. In a separate experiment, the order of the coinfusion solutions was reversed. All infusion rates were maintained at 19.4 μl/min. Blood samples of 0.5 ml were drawn through the jugular vein throughout the study, centrifuged and the plasma was immediately stored until HPLC analysis.

Product Inhibition Study

Male Sprague-Dawley rats (320 ± 5 g) were implanted with jugular vein and femoral vein cannulas under light ether anesthesia, one day prior to the study. A loading dose of 15 mg *N*-(2-hydroxyethyl) nicotinamide or 7.2 mg niacinamide (prepared in 0.9% saline) was administered over 1.27 min at 0.786 ml/min through the jugular vein. Immediately following the loading dose, the jugular cannula was flushed with saline. At the same time as the loading dose was started, a 7.88 mg/hr (1.0 ml/hr) maintenance dose of *N*-(2-hydroxyethyl) nicotinamide or 3.3 mg/hr (1.0 ml/hr) niacinamide was initiated through the femoral vein and was continued for 5 hr. This dosing regimen was intended to produce plasma concentrations of *N*-(2-hydroxyethyl) nicotinamide or niacinamide at about 15 μg/ml. After one hour of metabolite infusion, a 0.75 mg nicorandil bolus dose was administered through the jugular vein cannula. Blood samples were drawn at -0.5, 0.5, 1.083, 1.25, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 hr before and following the start of the infusion and centrifuged. The plasma was stored at -20°C until HPLC analysis. Control studies were conducted with a loading dose and infusion of 0.9% saline with subsequent 0.75 mg nicorandil bolus dosing, as described above.

Pharmacokinetic Modelling

Preliminary model fitting using the i.v. bolus data: The i.v. bolus data were used for preliminary model fitting to determine the basic pharmacokinetic model needed for further refinement. Parameter estimates obtained from these preliminary efforts were used to provide computer simulated values of concentration-time points from the various rates of infusion. These simulated values were then compared to the observed values by visual inspection to provide a gauge of the appropriateness of the basic pharmacokinetic model.

Three basic pharmacokinetic models were selected for this phase of the work. The first model which was tested was a simple Michaelis-Menten model with parallel first-order elimination. This model was examined because the concentrations obtained from the infusions appeared to approach apparent steady-state independently of the infusion rate, which is consistent with this model. The independent approach to steady-state could be attributed to a parallel first-order elimination component.

$$-\frac{d[C]}{dt} = \frac{V_{max}[C]}{[C] + K_m} + k_{el}[C] \qquad (eq.\ 1)$$

In eq. 1, -d[C]/dt is the rate of decline of nicorandil, K_m and V_{max} are the Michaelis-Menten parameters related to nicorandil metabolism, and k_{el} is the parallel first-order elimination rate constant for nicorandil.

The second model chosen for evaluation was that of cosubstrate depletion. The rationale for this model lies in the observation that the pharmacokinetics of nicorandil demonstrated features consistent with cosubstrate depletion, namely, an increase in the nicorandil dose brought about a disproportionate increase in plasma concentrations, concurrent with a dose-dependent increase in $t_{1/2}$ and monoexponential elimination (12,13). It has also been proposed that organic nitrate metabolism involves the oxidation of a critical sulfhydryl cofactor (14,15) before pharmacological action can be initiated. It is therefore possible that increasing doses of nicorandil may lead to depletion of this sulfhydryl cofactor.

$$-\frac{d[C]}{dt} = \frac{V_{max}[C][CS]}{K_m + [C][CS]} \qquad (eq.\ 2)$$

In equation 2, -d[C]/dt is the rate of decline of plasma nicorandil, K_m and V_{max} are the Michaelis-Menten parameters relating to nicorandil metabolism, and [CS] is the concentration of cosubstrate at the time of blood sampling. For ease of computation, only one cosubstrate was assumed to be relevant and that this cosubstrate had an identical molecular weight to nicorandil. (Any other molecular weight could also be assumed with the addition of a scaling factor.) The initial [CS] was arbitrarily set to the highest nicorandil concentration observed (60 μg/ml), and one molecule of CS was assumed to be utilized with each molecule of nicorandil metabolized.

The third model chosen for the preliminary i.v. bolus fitting was that of competitive product inhibition. The rationale for choosing this model was similar to that for the cosubstrate depletion model, that is, for an increase in dose, there appeared to be an apparent increase in $t_{1/2}$, even though the elimination curves for both i.v. bolus and i.v. infusion remained mono-exponential in nature (12,13,16). In addition, nicorandil is known to be biotransformed to several metabolites (8) which may compete for the same enzyme which mediates nicorandil metabolism. The following equation for competitive product inhibition was rearranged from Segel (17).

$$-\frac{d[C]}{dt} = \frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)} \qquad (eq.\ 3)$$

In eq. 3, -d[C]/dt is the rate of decline of nicorandil, K_m and V_{max} are the Michaelis-Menten parameters related to nicorandil metabolism, K_p is the product inhibition constant, and [P] is the concentration of the inhibitory product. For preliminary computer fitting of the i.v. bolus data, [P] was approximated as f([C_0]-[C]) where [C_0] was the nicorandil concentration at zero time and f represented the fraction of the metabolite(s) which was inhibitory.

Modifying the competitive product inhibition model: During this phase of the work, both the i.v. bolus and i.v. infusion data were fitted simultaneously. In order to improve the product inhibition model, additional elimination terms were added to the parent and metabolite equations (Table I). Model A is the classic competitive product inhibition model (17). Model B was derived by Perrier *et al.* (16) to describe the pharmacokinetics of a drug with competitive product inhibition, wherein the product was eliminated via first-order kinetics. Models C and D were derived in this study and they incorporated additional elimination terms (when compared to model B) in order to describe further nonlinear (model C) or first-order elimination of nicorandil (model D).

In Table I, -d[C]/dt is the rate of decline of nicorandil, K_m and V_{max} are the Michaelis-Menten parameters related to nicorandil metabolism, K_p is the product inhibition constant, [P] is the concentration of inhibitory product formed, k_{el1} and k_{el2} are the first-order elimination rate constants for

Table 1. Competitive product inhibition model refinements

MODEL	NICORANDIL $\frac{d[C]}{dt} =$	METABOLITE $\frac{d[P]}{dt} =$
MODEL A (17)	$-\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)}$	$\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)}$
MODEL B (16)	$-\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)}$	$\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)} - k_{el1}[P]$
MODEL C	$-\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)} - \frac{V_{mm}}{1 + \frac{K_{mm}}{[C]}}$	$\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)} - k_{el1}[P]$
MODEL D	$-\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(1 + \frac{[P]}{K_p}\right)} - k_{el2}[C]$	$\frac{V_{max}}{1 + \frac{K_m}{[C]}\left(\frac{1 + [P]}{K_p}\right)} - k_{el1}[P]$

the elimination of product and parent, respectively and V_{mm} and K_{mm} are the Michaelis-Menten parameters related to an additional nonlinear elimination pathway for parent. These additional pathways of elimination are assumed to be unaffected by product inhibition.

Statistical Analysis and Computer Fitting

Data are presented as mean ± S.D. Statistics on the i.v. bolus and i.v. infusion data were performed using a Student's t-test and one-way analysis of variance, respectively, with $p<0.05$ denoting statistical significance. Computer fitting of the i.v. bolus and infusion data was performed using an IBM 3090 and model 200J computer utilizing NONLIN 74 software. Pharmacokinetic simulations were performed using PCNONLIN (Statistical Consultants, Lexington, KY).

The NONLIN 74 program was capable of simultaneously fitting up to 20 differential equations. For preliminary fitting of the Michaelis-Menten with parallel first-order, cosubstrate depletion, and product inhibition models, only the i.v. bolus data was used, therefore data from all 9 i.v. bolus doses were used for the fitting. For further refinement of the product inhibition model, (fitting of models A-D), it was not possible to input all bolus injection and infusion data simultaneously for one combined fit. Therefore, only selected data sets (which covered the dose-ranges studied) were used in this exercise; the bolus doses chosen were 0.75, 1.5, 2, 3, 10 and 12 mg, and infusion doses of 10, 25, 50, 100 and 500 μg/kg/min, where $n=3$ for each dose. To describe the product concentration-time profile, initial values for the product were set to zero, and simulated values were generated. The half-lives were obtained by linear regression of the data.

The criteria used for arriving at the best model included the following: sums of squared deviations (S.S.D.), Akaike's Information Criterion (AIC) (18), Schwarz criterion (19) and visual inspection of the observed vs. predicted curves. After arriving at a satisfactory fit, we used the parameter estimates provided by the best fit, and simulated the concentration vs. time data from all i.v. bolus and infusion doses, and compared them to the observed data. For preliminary model fitting, the initial estimate for V_m was arbitrarily chosen as 10 times the highest infusion rate, K_m was arbitrarily chosen as 10 times the highest concentration observed for the infusion data, and K_p was arbitrarily set to be one-tenth the K_m. The parameter values from the preliminary model fitting of the competitive product inhibition model were used as initial estimates for the modified product inhibition model fitting. The parameter values finally obtained from the model fitting were found to be insensitive to 10-fold changes in initial estimates.

RESULTS

The observed nicorandil plasma concentrations after various i.v. bolus and i.v. infusion doses are shown in Fig. 1. After bolus doses (left panel), there was a suggestion of a short distribution phase, followed by monoexponential decline. The apparent elimination $t_{1/2}$ increased with increasing dose (Table II), and the apparent clearance decreased 5-fold following a 16-fold increase in bolus dose (Table II). Comparison of the apparent clearance at 0.75 and 3 mg bolus doses (0.289 ± 0.017 L/hr, $n=3$ and 0.167 ± 0.028 L/hr, $n=4$, respectively) showed these values to be statistically different ($p<0.05$), indicating the presence of nonlinear pharmacokinetics. Similar dose-dependent changes in the appar-

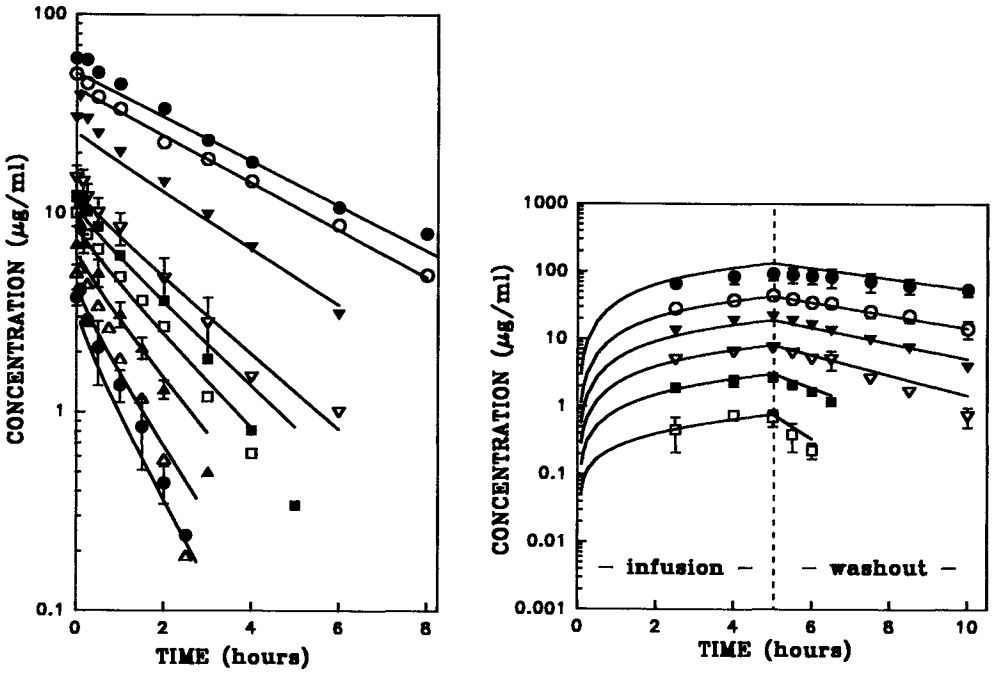

Figure 1. Plasma nicorandil concentration vs. time data after i.v. bolus doses of 0.75-12 mg, (left panel, $n=1-4$ at each dose) and after i.v. infusion of 10-500 μg/kg/min, (right panel, $n=3$ at each dose). Symbols represent experimental data and lines represent values calculated using the modified product inhibition model (model D).

Table II. Pharmacokinetic parameters for nicorandil i.v. bolus and infusion doses

Bolus dose (mg)	N	$t_{1/2}$ (hr)	C_0 (μg/ml)	V_d (ml)	k (hr^{-1})	$AUC_{0-\infty}$ (μg × hr/ml)	Cl (L/hr)
0.75	3	0.7 ± 0.1*	4.0 ± 0.4	191 ± 20**	1.10 ± 0.10	2.6 ± 0.2	0.289 ± 0.017*
1.0	1	0.5	6.6	152	1.30	4.7	0.215
1.5	3	0.7 ± 0.0	9.3 ± 2.0	165 ± 34	0.98 ± 0.02	7.9 ± 2.0	0.199 ± 0.050
2.0	1	1.0	12.0	167	0.70	11.6	0.172
2.5	1	1.0	12.9	194	0.69	17.2	0.146
3.0	4	1.2 ± 0.3*	15.4 ± 2.0	206 ± 31**	0.60 ± 0.16	21.1 ± 7.0	0.167 ± 0.028*
6.0	1	1.7	40.0	150	0.40	56.5	0.106
10.0	1	2.5	43.9	228	0.28	100	0.099
12.0	2	2.6, 2.6	60.0, 76.2	200, 157	0.26, 0.26	300, 162	0.040, 0.057

Infusion (μg/kg/min)	N	$t_{1/2}$† (hr)	C_{5h} (μg/ml)	C_{5h}/k_0 (ml/hr)
10	3	0.6 ± 0.1	0.7 ± 0.2	4.1 ± 0.2
25	3	1.2 ± 0.1	2.8 ± 0.5	2.9 ± 0.3
50	3	1.5 ± 0.2	7.4 ± 1.1	2.2 ± 0.1
100	3	2.1 ± 0.2	21.7 ± 1.9	1.4 ± 0.0
200	3	3.2 ± 0.8	44.4 ± 2.3	1.4 ± 0.1
500	3	6.2 ± 0.9	93.1 ± 19.0	1.8 ± 0.3

N is the number of animals investigated; C_0 is the concentration of nicorandil at time zero; V_d is the volume of distribution; k is the elimination rate constant; clearance, CL, was calculated as dose/$AUC_{0-\infty}$, where $AUC_{0-\infty}$ is the area under the plasma concentration vs. time curve from time zero to infinity; C_{5h} is the concentration of nicorandil at 5 hours of infusion.
* $p < 0.05$; **not significantly different, when comparing the same parameter at 0.75 and 3 mg doses, using an unpaired t-test; †one-way analysis of variance, $t_{1/2}$ dependent on infusion rate, $p < 5 \times 10^{-6}$.

ent elimination $t_{1/2}$ were observed when the infusions were terminated (Table II, and Fig. 1, right panel); one-way ANOVA showed this parameter to be highly dependent on the infusion rate ($p<5\times10^{-6}$). Likewise, the C_{5h}/k_0 (concentration at 5 hr normalized for the infusion rate) decreased 2-fold with a 50-fold increase in infusion rate (Table II). The apparent time to steady-state after various infusion doses was, however, relatively insensitive to changes in dose.

Preliminary model fitting of the i.v. bolus data to equations 1-3 indicated that the product inhibition model (eq. 3) was better than the other two models in describing the data on nicorandil pharmacokinetics (Fig. 2). The S.S.D., AIC and Schwarz values for the competitive product inhibition model were 162, 400 and 410, respectively. These test criteria were considerably less than those found for eq. 1 (354, 518, and 526, respectively) and for eq. 2 (194, 436 and 445, respectively).

However, eq. 3 still did not describe the data adequately (Fig. 2C). When modifications to eq. 3 were added (Table I, models A-D), the fits improved. The resulting parameter estimates and statistical tests are shown in Table III. For models A and B, the simulations did not describe the i.v. bolus data well, especially at the lowest i.v. doses, and the simulated infusion data did not match the observed values well at the lowest and highest doses. Model C, in which an additional Michaelis-Menten elimination term was incorporated for the parent drug, demonstrated some improvement over models A and B as judged by visual inspection of the simulations and by the lower S.S.D., AIC and Schwarz test values (Table III). Yet model C poorly predicted the lowest and highest i.v. infusion and bolus doses (data not shown).

The observed data were compared with simulated values of plasma concentrations vs. time using model D (Table I) in which the elimination of nicorandil was described by simultaneous Michaelis-Menten kinetics with product inhibition and first-order elimination (Fig. 1). Use of this kinetic model resulted in a better description of both the i.v. data sets as judged by visual inspection, and by lower S.S.D., AIC and Schwarz values (Table III).

Brief pharmacokinetic studies were then conducted in an attempt to validate the pharmacokinetic model with experimental data. The effect of a sulfhydryl donor, *N*-acetyl-L-cysteine, on the pharmacokinetics of nicorandil infusion was examined. Fig. 3 shows that a 3-hr coinfusion of nicorandil (200 μg/kg/min) and *N*-acetyl-L-cysteine (6.47 mg/kg/min) did not produce any difference in nicorandil pharmacokinetics from those obtained from a 3-hr coinfusion of nicorandil (200 μg/kg/min) and *N*-acetylserine (the nonsulfhydryl analog of *N*-acetyl-L-cysteine, also at 6.47 mg/kg/min) in the same rats (n = 2).

An experiment was conducted to determine whether *N*-(2-hydroxyethyl) nicotinamide was an inhibitor of nicorandil clearance. A dosing regimen (see Methods) of this metabolite was devised which aimed at achieving a steady-state concentration of *N*-(2-hydroxyethyl) nicotinamide at 15 μg/ml, which was the concentration observed for this metabolite after 5 hours of nicorandil infusion at 500 μg/kg/min. Results in Fig. 4 show that this objective was generally met, since the concentrations of *N*-(2-hydroxyethyl) nicotinamide were found to be between 18-23 μg/ml throughout the experiment. The pharmacokinetics of nicorandil were apparently affected by the coinfusion of *N*-(2-hydroxyethyl) nicotinamide. The elimination $t_{1/2}$ increased from 0.67 ± 0.05 hr (n = 5) in control animals to 1.13 ± 0.13 hr (n = 3) in the

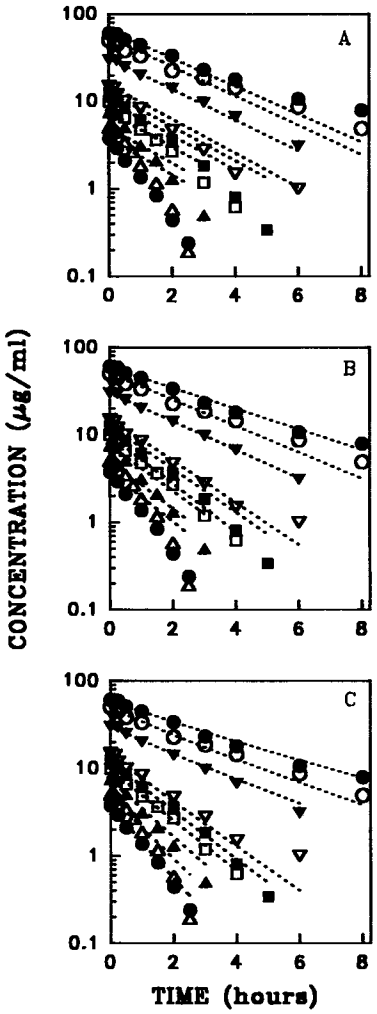

Figure 2. Comparison of three pharmacokinetic models fits to the observed nicorandil plasma concentrations after various bolus doses. The points represent observed data and the dashed lines represent the computer generated best fit line from preliminary model fitting, using equation 1, Michaelis-Menten with parallel first-order (panel A), equation 2, cosubstrate depletion (panel B), and equation 3, competitive product inhibition (panel C).

presence of the metabolite ($p<0.05$). The clearance was similarly altered, from 0.24 ± 0.05 L/hr in control rats to 0.17 ± 0.03 L/hr in metabolite-treated animals ($p<0.05$). Niacinamide, a nicotinamide analog, was also studied to determine whether it too would decrease the elimination of nicorandil. A similar steady-state concentration of 15 μg/ml niacinamide was targeted. The elimination $t_{1/2}$ increased from 0.65 ± 0.05 hr ($n=4$) in control animals to 1.02 ± 0.14 hr ($n=4$, $p<0.05$), in the presence of niacinamide (Fig. 5). The clearance was similarly altered from 0.22 ± 0.03 L/hr in control rats to 0.15 ± 0.03 L/hr in niacinamide treated animals ($p<0.05$).

DISCUSSION

Results of this study showed the pharmacokinetics of nicorandil in rats to be dose-dependent, after intravenous bolus doses of 0.75 to 12 mg, and 5-hour infusion doses of 10-500 μg/kg/min. The nonlinear pharmacokinetics appeared to be quite complex, and a modified product inhibition pharmacokinetic model needed to be invoked to describe the data. This model (model D) differed from previous literature models (16,17) in that linear elimination terms had to be added to the Michaelis-Menten functions to describe both the systemic elimination of the parent compound and the inhibiting metabolite.

The choice of the more complex pharmacokinetic model was supported on statistical grounds, based on traditional criteria (SSD, AIC and Schwarz criterion). In addition, key experimental evidence can be shown to be consistent with the proposed pharmacokinetic model. First, it is known that nicorandil undergoes metabolism in animals and humans, through a number of parallel pathways. The major pathway for nicorandil metabolism is through denitration. There are several minor pathways which account for the remaining nicorandil metabolism. These minor pathways include the formation of nicotinic acid, nicotinuric acid, nicotinamide and *N*-methyl nicotinamide (8,20). Thus, the use of multiple elimination terms to describe the disappearance of nicorandil was well justified. Second, we showed that the denitrated metabolite of nicorandil, *N*-(2-hydroxyethyl) nicotinamide, indeed inhibited the systemic clearance of the parent compound (Fig. 4).

Although literature data indicated that the pharmacokinetics of other organic nitrates need not be described by a similar product inhibition model, the concept of metabolite inhibition in the clearance of organic nitrates is not new. We have previously observed (21,22) that the systemic clearance of isosorbide dinitrate was inhibited when its denitrated metabolites, isosorbide-2-mononitrate and isosorbide-5-mononitrate, were exogenously administered. This metabolite-mediated inhibition might be responsible for the accumulation of isosorbide dinitrate in the plasma of angina patients upon chronic dosing (23). Cossum and Roberts (24) also observed a decrease in nitroglycerin degradation rate in whole blood when one of the dinitrate metabolites was added, and suggested competitive product inhibition as a mechanism for the concentration-dependent degradation kinetics of nitroglycerin in blood. Chong and Fung (13) confirmed this suggestion, and showed that concentration-dependent degradation was also observed for glyceryl dinitrates, but no glyceryl-1-mononitrate, in human blood. These authors suggested that product inhibition of nitrate degradation in human blood (13) required the metabolite to possess a nitrate group.

In the present case, however, we have shown nicorandil clearance to be affected by non-nitrate metabolites, such as *N*-(2-hydroxyethyl) nicotinamide (Fig. 4) and niacinamide (Fig. 5). Since both compounds contain the pyridyl ring in their structures, it appears likely that this functional group is important in mediating the binding and the subsequent metabolism of nicorandil. It is presently unknown which enzymes are primarily responsible for the metabolic clearance of organic nitrates, but the glutathione-S-transferases are probably involved (25,26). Since pyridyl containing compounds such as NADH and NADPH can participate in the glutathione oxidation pathway (27), it is possible that organic nitrates that carry nicotinamide in their structures might encounter more complex interactions in their metabolism. This

Table III. Table of parameter values (±S.D.) and statistical tests for models A, B, C and D

MODEL	S.S.D.	V_{max} μg/ml × hr	K_m μg/ml	K_p μg/ml	V_d ml	k_{el1} hr^{-1}	k_{el2} hr^{-1}	V_{mm} μg/ml × hr	K_{mm} μg/ml	AIC	Schwarz
A	16035	28.8 ± 3.5	41.5 ± 2.1	22.2 ± 3.4	241 ± 8					736	749
B	16007	28.3 ± 8.8	40.6 ± 12.3	22.1 ± 3.4	242 ± 11	1.6 ± 0.9 ×10^{-3}				737	753
C	15399	122.1 ± ***[a]	146.8 ± ***	8.4 ± ***	233 ± ***	0.16 ± ***		3.4× 10^{-7} ± ***	154.7 ± ***	715	737
D	5416	5.8 ± 1.1	1.2 ± 0.6	0.5 ± 0.4	237 ± 6	0.01 ± 0.02	0.14 ± 0.01			534	548

[a] *** denotes the inability of the computer to arrive at a value.
Data are expressed as parameter estimate ± S.D. of the parameter estimate.

might explain why product inhibition pharmacokinetics are readily seen with nicorandil, but are not apparent with other organic nitrates.

In our experiment, we administered *N*-(2-hydroxyethyl) nicotinamide to achieve a steady-state concentration of 15 μg/ml. This concentration was similar to that observed at the highest nicorandil infusion dose (500 μg/kg/min). Although this concentration of metabolite indeed inhibited nicorandil clearance by about 40% at an i.v. bolus nicorandil dose of 0.75 mg, the magnitude of inhibition is relatively small. However, it is recognized that the degree of inhibition from exogenously administered metabolites may be considerably smaller than that produced by endogenously formed metabolites (16), since the localized concentration at the enzyme site could be substantially higher in the latter case.

Chong and Fung (13) showed that, besides product inhibition, a pharmacokinetic model involving cosubstrate depletion can also produce linear elimination curves in the presence of dose-dependency. Since sulfhydryl groups have been well known to participate in the metabolism of organic nitrates (14,28), we administered a sulfhydryl donor, *N*-acetyl-L-cysteine, to determine whether the pharmacokinetics of nicorandil might be altered. We have chosen a *N*-acetyl-L-cysteine dose, 6.47 mg/kg/min, that had been shown to enhance the pharmacologic action, and therefore the intracellular metabolism, of nitroglycerin, the prototype organic nitrate (28). This *N*-acetyl-L-cysteine dose represented a molar excess of 42 fold when compared to the nicorandil dose. In limited studies involving only two animals (Fig. 3), we saw no evidence that the systemic pharmacokinetics of nicorandil was altered by coadministration of *N*-acetyl-L-cysteine. These results, limited though they were, argued against sulfhydryl cosubstrate depletion as a likely mechanism for the dose-dependent pharmacokinetics of nicorandil. Of course, it is possible that other cosubstrates at yet higher concentrations may produce different results.

The present findings may provide some implications on the clinical use of nicorandil and its analogs. Tice *et al.* (9) commented recently that "the rapid clearance and short pharmacokinetic-pharmacodynamic $t_{1/2}$ of nicorandil are not favorable features for long-term medical management of chronic heart failure". These authors also showed, however,

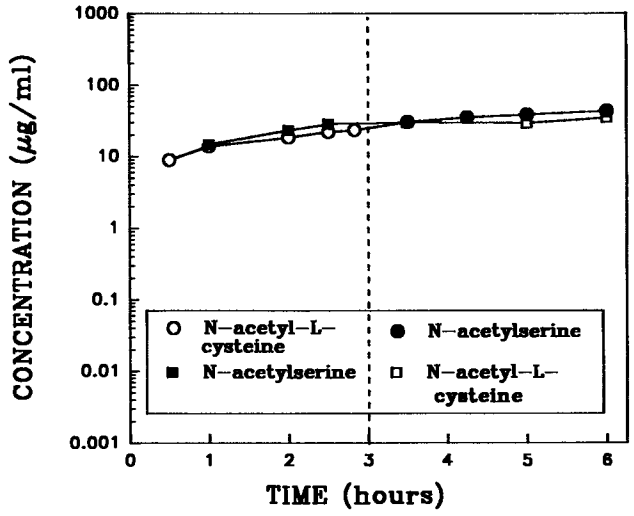

Figure 3. Plasma concentration vs. time profile for nicorandil + *N*-acetylserine and nicorandil + *N*-acetyl-L-cysteine infusions (n = 2). ○, *N*-acetyl-L-cysteine + nicorandil followed by ●, *N*-acetylserine + nicorandil; ■, *N*-acetylserine + nicorandil followed by □, *N*-acetyl-L-cysteine + nicorandil.

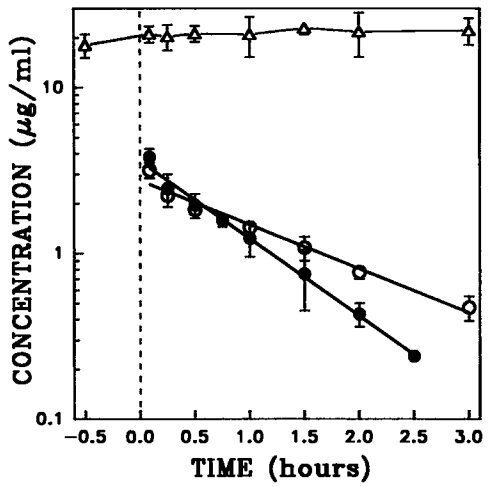

Figure 4. Nicorandil plasma concentrations after a 0.75 mg bolus dose with and without co-infusion of metabolite, *N*-(2-hydroxyethyl) nicotinamide. ●, control (nicorandil only, n = 5). ○, nicorandil with coinfusion of *N*-(2-hydroxyethyl) nicotinamide at 1.0 ml/hr, (n = 3). △, *N*-(2-hydroxyethyl) nicotinamide concentration (n = 3). The lines were obtained from linear regression.

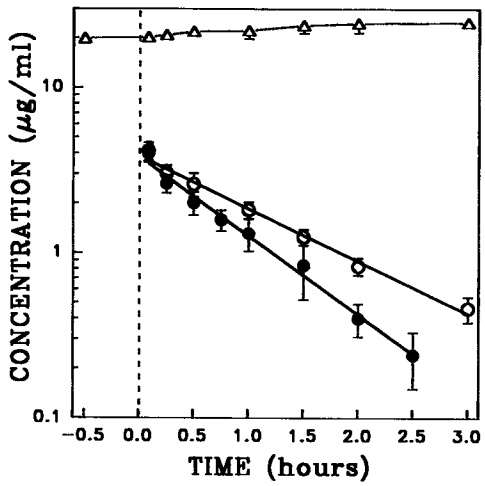

Figure 5. Nicorandil plasma concentrations after a 0.75 mg bolus dose with and without co-infusion of niacinamide. ●, control, (nicorandil only, n = 4). ○, nicorandil with coinfusion of niacinamide at 1.0 mg/hr, (n = 4). △, niacinamide concentration (n = 4). The lines were obtained from linear regression.

that in patients with congestive heart failure who were given the drug orally at 10 to 60 mg, the apparent elimination half-life of nicorandil increased with dose. These clinical findings are consistent with the present results, and suggest that the pharmacokinetics and pharmacodynamics of nicorandil may possibly be manipulated by changing the dosing regimen (to produce different profiles of the inhibiting metabolite(s)), or by coadministering relatively nontoxic compounds such as niacinamide to decrease nicorandil clearance. Nicorandil analogs with substituted pyridyl ring structures have been prepared and shown to be potent vasodilators (4,29–31). It will be interesting to determine whether the pharmacokinetics of these analogs are similarly affected by product inhibition.

The modified product inhibition pharmacokinetic model described in this report bears some interesting characteristics. For example, as the dose was increased, either as a bolus injection or infusion, the apparent elimination curve remained linear, though dose-dependent. The rate of approach to apparent steady-state concentration of nicorandil did not seem to be overtly dependent on the infusion rate, even though over the same range of infusion rates, the elimination half-life was clearly dose-dependent. It is possible that this model may be applicable for the description of the pharmacokinetics of other drugs that are subject to product inhibition.

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