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# Pharmacokinetics of Nicorandil, a New Coronary Vasodilator, in Dogs

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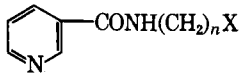
**Abstract** □ The kinetic disposition of nicorandil, *N*-[2-(nitroxy)ethyl]-3-pyridinecarboxamide (I), and its main metabolic product, *N*-[2-(hydroxy)-ethyl]-3-pyridinecarboxamide (II), was studied after administering intravenous and oral doses (2.5 mg/kg) of nicorandil to the same beagle dogs. The plasma concentrations were measured using a high-performance liquid chromatographic method. The pharmacokinetic data derived from intravenous administration of nicorandil were: $t_{1/2}$, 0.73 ± 0.11 h; $Vd_{area}$, 0.67 ± 0.04 L/kg; and total plasma clearance, 13.50 ± 1.05 mL/min/kg. After oral administration, nicorandil was rapidly absorbed ($t_{max}$, 0.58 ± 0.11 h). The oral bioavailability was calculated as 0.72 ± 0.07. The metabolic formation of the corresponding alcohol after intravenous and oral administration of the parent compound appeared to occur quite efficiently, and its elimination half-life (3.09 ± 0.25 and 3.69 ± 0.88 h after intravenous and oral administration of nicorandil, respectively) was longer than that of the parent compound. Since the dose employed in this study was much higher than the expected therapeutic doses, whether such a good bioavailability after a lower dose of the drug would be obtained in humans remains unanswered.

**Keyphrases** □ Nicorandil—metabolism in dogs, pharmacokinetics, detection in plasma by HPLC □ Vasodilators, coronary—nicorandil, metabolism in dogs, pharmacokinetics, detection in plasma by HPLC □ Bioavailability—nicorandil in dogs, oral and intravenous administration, pharmacokinetics, detection in plasma by HPLC

Nicorandil[1] (*N*-[2-(nitroxy)ethyl]-3-pyridinecarboxamide, I) a new coronary vasodilator has been shown to produce a potent coronary vasodilating effect, virtually without affecting cardiac contraction, heart rate, or myocardial oxygen consumption in anesthetized dogs (1–3). The pharmacological profile appears to be somewhat similar to that of nitroglycerin (2–4). However, except for one study where the metabolic disposition of nicorandil was qualitatively examined in rats (5), the pharmacokinetics and bioavailability of this drug have not been investigated. The lack of a sensitive and specific method for measurement of nicorandil in the blood has precluded any quantitative pharmacokinetic analysis.

In this study we describe a high-performance liquid chromatographic (HPLC) method for determining nicorandil levels in canine plasma. Using this method, we attempted to study the kinetic disposition of nicorandil (I) administered intravenously and orally on two separate occasions in the same dogs. Some pharmacokinetic variables of *N*-[2-(hydroxy)-ethyl]-3-pyridinecarboxamide (II), a primary metabolite of nicorandil, were also estimated.



I X = $ONO_2$, $n$ = 2
II X = OH, $n$ = 2
III X = $ONO_2$, $n$ = 3

## EXPERIMENTAL

**Materials and Procedures**—Six adult beagle dogs weighing 8.2–9.9 kg were used for the study. They were fasted overnight; water was supplied *ad libitum*.

[1] The generic name was published in the *Supplement to WHO Chronicle*, **34**(9), 18 (1980). Lot R9B09, synthesized at the Organic Chemistry Research Laboratory, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan, was used throughout.



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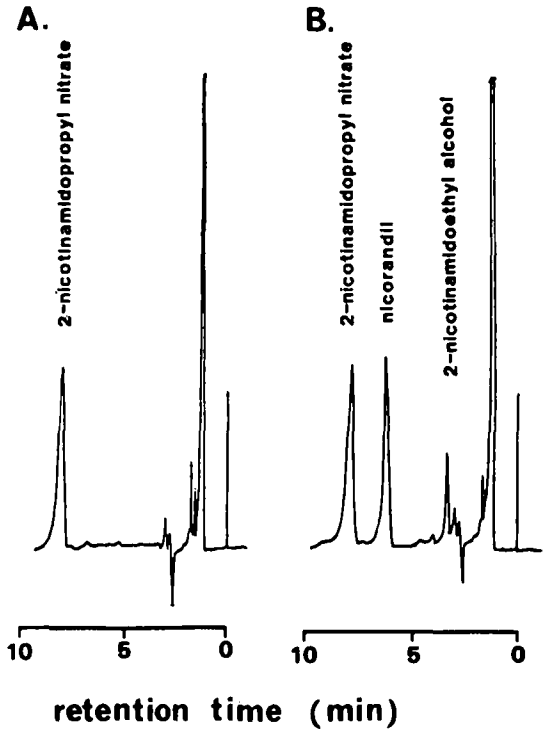


**Figure 1**—*Chromatograms of 10-μL canine plasma extracts. Key: (A) blank plasma spiked with 1.0 μg/mL of III (internal standard); (B) plasma spiked with 1 μg/mL of nicorandil and each of its analogues.*

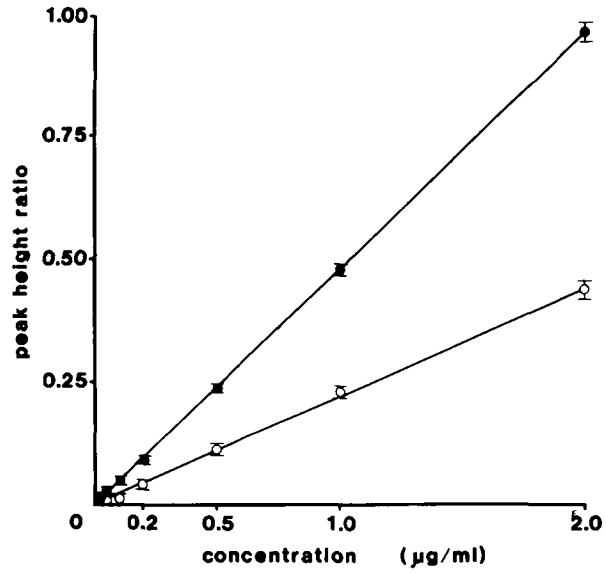


**Figure 2**—*Calibration curves for nicorandil and alcohol II in canine plasma. A four-point standard curve was prepared by plotting the ratio of the peak height of each compound to that of the internal standard, as mean ± SEM. Linear regression analysis of calibration curve data indicated no significant deviation from linearity for both compounds:* y = 0.4768x + 0.0031, r = 0.9997 *for nicorandil (●);* y = 0.2225x + 0.0006, r = 0.9970 *for alcohol II (○). In addition, intercept values did not significantly differ from zero.*

The dogs were examined during a 7-d acclimation period prior to the experiment, and showed no apparent hepatic or renal dysfunction. No anesthetic was administered during the experiments.

The dogs received 2.5 mg/kg of nicorandil (I) intravenously and orally on two occasions, separated by a 2-week wash-out period. The study was carried out in a randomized crossover fashion: three dogs received the intravenous dose first, while the oral dose was administered to the remaining three; the group given the intravenous dose in the first experiment was given the oral dose in the second and *vice versa*. Nicorandil was dissolved at a concentration of 10.0 mg/mL in 0.9% NaCl for intravenous injection (infused into the antebrachial vein over 30 s) or in distilled water for oral administration. The solutions were prepared just prior to administration. Oral dosing was accomplished with the aid of a rubber catheter (6.5 mm i.d., 9.5 mm o.d.) which was inserted ~40 cm through the mouth into the esophagus.

Blood samples (5 mL) for drug assay were withdrawn into heparinized syringes from the antebrachial vein opposite to where nicorandil was intravenously administered prior to and at 1, 2, 4, 10, 15, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, and 12 h after administration by either route. Immediately after blood collection, the plasma was separated and stored at −20°C until assayed.

**Assay Method**—The nitrate III[2] was used as the internal standard. Two milliliters of the plasma was transferred to a 15-mL stoppered conical-tip glass tube to which 1 mL of distilled water, 1 mL of internal standard solution (1 mL of distilled water contained 2.5 μg of internal standard III), two drops of 1 M NaOH, and 6.5 mL of ethyl acetate were added. The mixture was vigorously shaken in a mechanical shaker for 10 min. The upper ethyl acetate layer was removed after centrifugation at 3 × 10 rpm for 10 min, and the extraction procedure was repeated. The ethyl acetate layers were combined, transferred to a 30-mL pear-shaped glass flask, and evaporated to dryness. Immediately after evaporation, 0.5 mL of distilled water was added to dissolve the residue.

Ten-microliter samples were injected into an HPLC system[3] with a 30-cm × 4-mm i.d. stainless steel column[4]. The flow rate of the solvent, 25% methyl alcohol and 7% ethyl alcohol in distilled water, was 1.1 mL/min; the operating temperature was 25 ± 2°C. Successive eluates were checked by means of a UV absorption detector (254 nm with 0.01 AUFS) and recorded on a chart recorder at a rate of 0.5 cm/min. Under these conditions, the retention times were 6.0, 3.5, and 8.0 min for I, II, and III, respectively (Fig. 1).

Standard curves for nicorandil (I) and alcohol II in canine plasma were prepared as follows. One milliliter of a solution containing the equal amounts of both compounds in distilled water and 1 mL of a solution of III (2.5 μg) were added to the control canine plasma (2.0 mL) to give duplicate standards. An equal volume of distilled water was added to the control plasma (blank). The prepared standard plasma samples were treated in the same manner as the test samples.

The calibration curves (Fig. 2) were linear, passing through the origin, at concentrations ranging from 0.025 to 4.0 μg/mL for I and from 0.2 to 4.0 μg/mL for II. The coefficients of a linear regression analysis of the calibration values were found to be 0.9997 for I and 0.9970 for II. The reproducibility of the analysis, as determined by quadruplicate assays of plasma with known concentrations of both compounds, was examined on different days over a 3-week period. The coefficients of variation found at concentrations of 0.5 and 2.0 μg/mL were 5.7 and 2.7% for I and 4.2 and 2.0% for II, respectively. Within-day assays gave the following coefficient of variation values for both compounds: 3.3 and 1.8% at concentrations of 0.5 and 2.0 μg/mL for I, respectively, and 9.7 and 3.8% at concentrations of 0.5 and 2.0 μg/mL for II, respectively. The recovery rate for nicorandil was 92.0 ± 4.1 (mean ± *SD*), 91.3 ± 2.7, 99.5 ± 3.5, and 98.0 ± 3.6% at concentrations of 0.5, 1.0, 2.0, and 4.0 μg/mL of canine plasma (from 10 determinations at each concentration); the value for II was quite low, yielding 13.2 ± 2.6, 12.6 ± 2.3, 14.3 ± 1.6, and 14.6 ± 1.9%, respectively, at the same concentrations as for I. The sensitivity limits were ~0.025 μg/mL for I and ~0.2 μg/mL for II.

The stability of the test compounds in frozen plasma was also examined. Standard solutions containing 1, 2, and 4 μg of I, II, and III, respectively, were added to 2.0-mL aliquots of canine plasma, and the mixtures were stored at −20°C; the respective compounds were assayed on days 18 and 40 of storage. The mean ±*SD* recovery rates on day 18 relative to the same standard solutions freshly prepared were 101.5 ± 3.7% for I, 94.8 ± 2.6% for II, and 96.0 ± 3.5% for III; the respective values for the three compounds on day 40 were 95.0 ± 4.5, 94.0 ± 3.7, and 94.7 ± 3.5%. These compounds are considered to be stable in plasma kept at −20°C for a period of ≥40 d.

Concentrations of I and II reported in this study were the values measured within 20 d after the blood collection. Duplicate analyses were done on all samples with the average values reported.

**Pharmacokinetic and Statistical Calculations**—Model-independent pharmacokinetic parameters were estimated throughout the study. The terminal elimination rate constant of nicorandil ($K$) and its metabolite ($K_{met}$) was calculated from the least-squares regression slope of the terminal log-linear phase of the phase concentration–time data. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2}$ or $t_{1/2met} = 0.693/K$ or $K_{met}$.

The area under the curve against time (AUC) for nicorandil was estimated

[2] Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

[3] The HPLC system consisted of a model U6K injector, a model 6000A high-pressure pump, and a model 440 absorbance detector, all from Waters Associates, Milford, Mass.

[4] μ Bondapak $C_{18}$; Water Associates, Milford, Mass.

**Table I—Pharmacokinetic Parameters of Nicorandil Following a 2.5-mg/kg iv Dose of Nicorandil**

| Dog | Weight, kg | $K$, $h^{-1}$ | $t_{1/2}$, h | $Vd_{area}$, L/kg | $CL_{tot}$, mL/min/kg | $AUC_0^\infty$, μg·h/mL |
|---|---|---|---|---|---|---|
| 1 | 8.8 | 0.56 | 1.23 | 0.67 | 12.37 | 3.37 |
| 2 | 8.2 | 1.54 | 0.45 | 0.67 | 17.76 | 2.35 |
| 3 | 9.1 | 0.93 | 0.74 | 0.73 | 12.93 | 3.22 |
| 4 | 9.3 | 1.29 | 0.54 | 0.59 | 14.00 | 2.98 |
| 5 | 9.2 | 0.89 | 0.78 | 0.82 | 14.01 | 2.97 |
| 6 | 9.9 | 1.08 | 0.64 | 0.56 | 9.94 | 4.19 |
| Mean | 9.1 | 1.05 | 0.73 | 0.67 | 13.50 | 3.18 |
| *SEM* | ±0.2 | ±0.14 | ±0.11 | ±0.04 | ±1.05 | ±0.25 |

**Table II—Pharmacokinetic Parameters of Nicorandil Following a 2.5-mg/kg Oral Dose of Nicorandil**

| Dog[a] | $K$, $h^{-1}$ | $t_{1/2}$, h | $C_{max}$, μg/mL | $t_{max}$, h | $AUC_0^\infty$, μg·h/mL | $F$ | $F'$ | $F'/F$[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.86 | 0.81 | 1.9 | 0.75 | 2.23 | 0.66 | 0.69 | 1.05 |
| 2 | 0.69 | 1.00 | 0.9 | 0.50 | 1.69 | 0.72 | 0.56 | 0.78 |
| 3 | 0.60 | 1.16 | 0.7 | 0.50 | 1.92 | 0.60 | 0.68 | 1.13 |
| 4 | 0.80 | 0.86 | 0.8 | 0.50 | 1.54 | 0.52 | 0.65 | 1.25 |
| 5 | 1.01 | 0.69 | 2.1 | 0.25 | 2.94 | 0.99 | 0.65 | 0.66 |
| 6 | 0.80 | 0.87 | 1.6 | 1.00 | 3.65 | 0.87 | 0.75 | 0.86 |
| Mean | 0.79 | 0.90 | 1.3 | 0.58 | 2.33 | 0.72 | 0.66 | 0.95 |
| *SEM* | ±0.06 | ±0.07 | ±0.2 | ±0.11 | ±0.33 | ±0.07 | ±0.03 | ±0.09 |

[a] Each number of animals is the same as that given in Table I. [b] The mean ± *SEM* value is 1.01 ± 0.09 when dog 5 is excluded.

according to $AUC_0^\infty = AUC_0^T + C_p^T/K$. The $AUC_0^T$ was calculated using the trapezoidal method, where $T$ is the time of the last measured plasma concentration of nicorandil ($C_p^T$). The apparent volume of distribution ($Vd_{area}$) of nicorandil was obtained from the equation, $Vd$ = intravenous dose/(intravenous $AUC_0^\infty \cdot K$). The apparent total body clearance ($CL_{tot}$) of nicorandil was calculated from $CL_{tot}$ = intravenous dose/intravenous $AUC_0^\infty$.

The absolute bioavailability of nicorandil ($F$) after oral administration was determined from $F$ = oral $AUC_0^\infty$/intravenous $AUC_0^\infty$, where the oral $AUC_0^\infty$ is defined as $AUC_0^T + C_p^T/K$. The maximal plasma nicorandil concentration ($C_{max}$) after oral administration and the time to peak concentration ($t_{max}$) were read from the concentration-time data of each animal.

We also predicted the theoretical value for oral bioavailability ($F'$) of nicorandil using the following equation (6): $F'$ = 1 − intravenous dose/(intravenous $AUC_0^\infty \cdot \lambda \cdot Q$), where $Q$ is the liver blood flow of 41.0 mL/min/kg in the dog (7) and λ is the blood-plasma concentration ratio of nicorandil in the dog, which is known to be close to unity (1.01 ± 0.05, $n$ = 10)[5]. This $F'$ value was compared with the calculated value ($F$) for each dog.

Such pharmacokinetic variables as $Vd_{area}$ and $CL_{tot}$ were normalized for the body weight of the animal and expressed as liters per kilogram and milliliters per minute per kilogram, respectively. All average data are given as the mean ± *SEM*. All the pharmacokinetic and statistical calculations were done with a computer[6]. Statistical comparison of the data obtained using the two different routes of drug administration was made by a paired Student's $t$ test, with a significance level of $p < 0.05$.

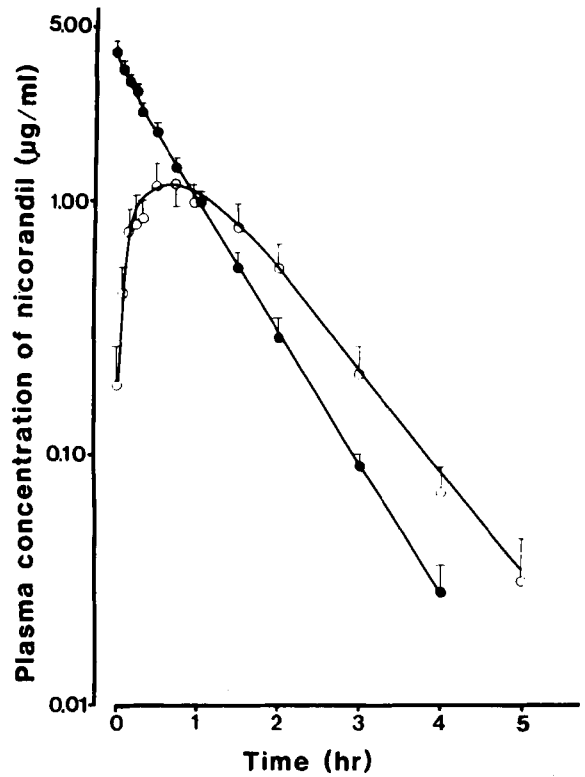


**Figure 3**—*Average concentration-time profile of nicorandil in plasma after intravenous (●) or oral (○) administration of a 2.5-mg/kg dose of nicorandil to six dogs, mean* ± SEM.

## RESULTS

Semilogarithmic plots of nicorandil plasma concentrations against time after intravenous and oral administrations of the same dose of nicorandil (2.5 mg/kg) are shown in Fig. 3. Pharmacokinetic parameters of nicorandil related to intravenous and oral dosing are summarized in Tables I and II, respectively.

Following intravenous administration, the elimination phase had a $t_{1/2}$ of 0.73 ± 0.11 h. No nicorandil could be detected in the plasma 5 h after dosage with this HPLC method. The $AUC_0^\infty$ values ranged from 2.35 to 4.19 μg·h/mL. The weight-normalized volume of distribution ($Vd_{area}$) and total body (plasma) clearance ($CL_{tot}$) was 0.67 ± 0.04 L/kg and 13.50 ± 1.05 mL/min/kg, respectively.

After oral administration, nicorandil in plasma was detected within 10 min of dosing and reached peak concentrations between 0.25 and 1.0 h ($t_{max}$, Table II). The mean peak plasma concentration ($C_{max}$) was 1.3 ± 0.2 μg/mL at ~0.58 h. After 6 h, no drug could be detected in the plasma with the HPLC method used. The interindividual variation in oral $AUC_0^\infty$ (≃2.4-fold) was larger than that of intravenous $AUC_0^\infty$ (≃1.8-fold) for the six dogs. The mean $AUC_0^\infty$ was statistically different ($p < 0.02$) for the two different routes of nicorandil administration. The mean bioavailability ($F$), calculated from oral $AUC_0^\infty$/intravenous $AUC_0^\infty$, was 0.72 (Table II). The value ($F'$) predicted the equation (6) was fairly consistent with that of $F$ in all but one dog (no. 5). This dog showed the greatest $C_{max}$ (2.1 μg/mL) with the shortest $t_{max}$ (0.25 h), which may seemingly diminish the hepatic first-pass effect for orally administered nicorandil. Except for this dog, the ratio of $F'/F$ ranged from 0.78 to 1.25 (mean = 1.01; Table II).

Semilogarithmic plots of plasma concentration-time data of alcohol II following intravenous and oral administration of nicorandil are shown in Fig. 4. The mean value for the oral $AUC_0^\infty$ (13.0 ± 1.7 μg·h/mL) was identical with that for the intravenous $AUC_0^\infty$ (13.5 ± 0.1 μg·h/mL). The mean terminal half-life of the metabolite (Table III) was much longer than that of the parent drug (Tables I and II).

[5] H. Kamiyama, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan, unpublished data.

[6] M100 Ace Mark IV; SORD Computer Systems, Inc., Tokyo, Japan.

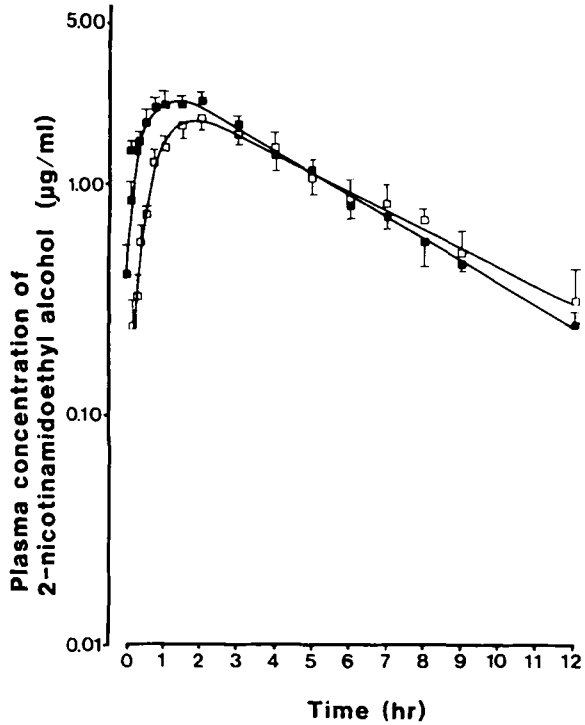


**Figure 4**—*Average concentration-time profile of alcohol II in plasma after intravenous (■) or oral (□) administration of a 2.5-mg/kg dose of nicorandil to six dogs, mean* ± SEM

## DISCUSSION

The elimination half-life observed for nicorandil in this study appears to be longer than the respective values of conventional coronary vasodilators such as glyceryl trinitrate (nitroglycerin), isosorbide dinitrate, and pentaerythritol trinitrate, which are on the order of minutes in animals (including dogs) and humans (8–10). Previous workers (9) noted a plasma half-life for unchanged isosorbide dinitrate of ~7 min in dogs, while others (10) observed a plasma half-life for nitroglycerin of 4.4 min in normal volunteers. A preliminary clinical study (11) showed that the mean elimination half-life observed in 10 patients with heart disease after a single 20-mg oral administration of nicorandil was ~52 min (≃0.87 h).

It is noteworthy that the fraction available in the systemic circulation (*F*) for nicorandil, a mononitrate compound, was ~70% with a long elimination half-life (0.90 ± 0.07 h) after oral administration to dogs (Table II). This seems to be in marked contrast to the di- and trinitrate compounds in various animal species, including dogs (8, 9, 12). Studies conducted in animals (8, 9, 13–16) and humans (17) have suggested that the di- and trinitrates are rapidly and extensively degraded in the liver. On the other hand, isosorbide-5-mononitrate, a major metabolite of isosorbide dinitrate in dogs (9, 12) and humans (18–20), has recently been reported to be completely bioavailable after a single oral dose of 20 mg (21); the elimination half-life of isosorbide-5-mononitrate is ~8 times that of isosorbide dinitrate (20, 22).

We are tempted to speculate that the mononitrate forms of coronary vasodilators (*e.g.*, nicorandil) are resistant to metabolic breakdown and will attain higher plasma concentrations, resulting in greater bioavailability after oral administration. As our bioavailability data on nicorandil were obtained from only one species, however, it must be interpreted with caution. There are several other agents whose systemic availability is not proportional to the dose administered. For example, single-oral-dose studies using propranolol (23) have shown that as large doses of the drug are ingested, the hepatic extraction becomes saturated, allowing more of the drug to reach the systemic circulation. Since the oral dose of nicorandil administered to the dogs was ~3.3- to 10-fold greater than that recently tested (5–15 mg/d TID) in patients with angina pectoris (24), whether a similar bioavailability after a lower dose of nicorandil would have been obtained remains unanswered; a dose-ranging pharmacokinetic study would be required. It is likely that the degradation rate of nicorandil may vary from species to species, and that the bioavailability data from an animal species can not directly be translated into human terms.

Concerning the formation of the main metabolic product of nicorandil, the alcohol II, the low recovery of this metabolite from canine plasma using this HPLC method hampers discussion on its quantitative disposition. Bearing this limitation in mind, however, the formation of this metabolite appears to occur quite efficiently in dogs after both intravenous and oral administrations of the parent compound (Fig. 4). The metabolite has a longer half-life (Table III) compared with the parent compound (Tables I and II), and plasma concentrations were maintained at higher levels for a longer period of time following intravenous or oral administration of nicorandil (Fig. 4). One may question if the metabolite would affect the disposition of the parent compound, thereby modifying the cardiovascular effects of nicorandil. However, previous workers (1, 3, 5) have demonstrated that alcohol II is pharmacologically inactive and has no influence on the cardiovascular effects of nicorandil. Nevertheless, whether the alcohol can distort the disposition of nicorandil remains totally unknown.

**Table III—Apparent Pharmacokinetic Parameters of Alcohol II Following a 2.5-mg/kg iv or Oral Dose of Nicorandil**

| Dog | Intravenous Study | | Oral Study | |
|---|---|---|---|---|
| | $K_{met}$, $h^{-1}$ | $t_{1/2met}$, h | $K_{met}$, $h^{-1}$ | $t_{1/2met}$, h |
| 1 | 0.18 | 3.76 | 0.26 | 2.68 |
| 2 | 0.28 | 2.50 | 0.23 | 3.05 |
| 3 | 0.20 | 3.43 | 0.34 | 2.05 |
| 4 | 0.22 | 3.15 | 0.10 | 6.93 |
| 5 | 0.31 | 2.18 | 0.36 | 1.94 |
| 6 | 0.20 | 3.50 | 0.14 | 4.90 |
| Mean | 0.23 | 3.09 | 0.24 | 3.60 |
| *SEM* | ±0.02 | ±0.25 | ±0.04 | ±0.80 |

In summary, nicorandil has a longer elimination half-life, and its oral bioavailability appears considerably greater than conventional nitrate compounds. However, before nicorandil becomes a therapeutic agent for the treatment of angina pectoris, its disposition and metabolic fate in the human body must be elucidated. Undoubtedly, this requires a more sensitive and specific assay method. Thus, the results reported herein using this HPLC method, which is sensitive enough to detect nicorandil only in the plasma of dogs administered a relatively large dose of nicorandil, should be viewed as a basis for further studies.

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# Isolation and Identification of Piperacillin Amide as an Impurity in Piperacillin

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**Abstract** □ Piperacillin amide (IV) was successfully identified as the predominant impurity in commercial lots of piperacillin monohydrate (III). The impurity was isolated *via* a preparative liquid chromatographic scheme utilizing Florisil as the adsorbent and a mobile phase of water–acetonitrile (4:96, v/v). The isolated component had nearly the same reverse-phase HPLC properties as piperacillin and was chemically and thermally unstable. This labile impurity was spectroscopically identified by field desorption (FD), fast atom bombardment (FAB) with collision activation decomposition (CAD), and desorption chemical ionization (DCI) mass spectrometries, and NMR and IR spectrometries. Identity was confirmed on comparison of the chromatographic and spectrometric data of the impurity with an independently synthesized sample of piperacillin amide.

**Keyphrases** □ Piperacillin amide—impurity in piperacillin, isolation, identification using soft-ionization mass spectra, high-field NMR, and IR spectrometry □ Piperacillin—isolation of an amide impurity by column chromatography, identification using soft-ionization mass spectra, high-field NMR, and IR spectrometry, antibiotic activity

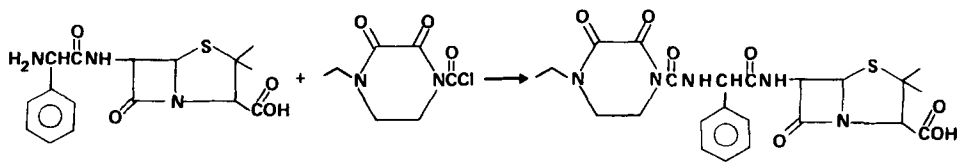
Piperacillin[1] is a third generation semisynthetic penicillin antibiotic with a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative organisms (1). As shown in Scheme I, piperacillin (III) is produced from the reaction of ampicillin (I) with 4-ethyl-2,3-dioxo-1-piperazinecarbonyl chloride (II). Five low-level components, each ≤0.6% w/w, were observed in the final piperacillin product. Two of these components were related to the starting materials: ampicillin and 1-ethyl-2,3-piperazinedione (2). Two components were expected process impurities: the β-lactam hydrolysis product of piperacillin (3) and the condensation product of piperacillin with ampicillin (2). The isolation and identification of the fifth component is the subject of this paper. This component, referred to as the "unknown piperacillin impurity," constituted the major impurity in commercial lots of piperacillin, 0.2–0.4% w/w[2]. This impurity proved to be difficult to isolate chromatographically and to characterize spectrometrically.

Two main factors which complicated the development of a reverse-phase HPLC method for isolating the unknown piperacillin impurity from piperacillin in quantities large enough for spectrometric analysis are:

1. the difficulty in resolving the major impurity from piperacillin because the impurity eluted as a shoulder on the high retention time side of piperacillin;
2. the chemical instability of the unknown material in normal HPLC reverse-phase solvents, *viz.*, methanol and aqueous media, at other than neutral pH for extended periods.

For these reasons, a unique preparative column chromatographic method using an isocratic normal phase was developed which was successful in resolving the unknown piperacillin impurity from piperacillin.

Identification of the isolated impurity was not routine, since it was highly polar and chemically and thermally unstable. The unknown impurity was characterized by mass spectrometry using soft-ionization conditions, *i.e.*, field desorption (FD) (4, 5), fast atom bombardment (FAB) (6, 7), and desorption chemical ionization (DCI) (8, 9). High-field NMR and Fourier transform IR spectrometries were also used to elucidate the structure of the unknown piperacillin impurity. The spectrometric data suggested that the unknown was piperacillin amide (IV). This hypothesis was confirmed by compar-



(I) (II) (III)

*Scheme I*

[1] Marketed by Lederle Laboratories in the U.S. as Pipracil and in Europe as Pipril.

[2] Penticillin (Toyama Chemicals Co., Ltd. brand of piperacillin marketed in Japan) also contains piperacillin amide, but the quantity is negligible.



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