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DETERMINATION OF NICAINOPROL, A NEW ANTIARRHYTHMIC AGENT, IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

YUKIO HORAI* and TAKASHI ISHIZAKI

Division of Clinical Pharmacology, Clinical Research Institute, National Medical Center, Toyama 1-21-2, Shinjuku-ku, Tokyo 162 (Japan)

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SUMMARY

A new method for the determination of nicainoprol in human plasma and urine has been developed. Nicainoprol and *p*-chlorodisopyramide as the internal standard are extracted into dichloromethane under basic conditions, and then evaporated to dryness. A reconstituted aliquot is injected onto a cyanopropyl column with an automatic high-performance liquid chromatographic system and quantitated using ultraviolet detection at 250 nm. The whole system-elapsed time to analyse a sample is ca. 10 min, and the detection limit using 1 ml of plasma is 15 ng/ml. Preliminary plasma and urinary concentration—time data from a healthy subject following an oral nicainoprol administration are reported. The assay method presented appears to be selective, and is of sufficient sensitivity, precision and accuracy to be applicable to the study of the pharmacokinetic behaviour of nicainoprol in humans.

INTRODUCTION

Nicainoprol, [1-nicotinyl-8-(3-isopropylamino-2-hydroxypropoxy)] tetrahydroquinoline, a newly developed antiarrhythmic agent, is effective against experimental [1] and clinical arrhythmias [2] and possesses similar electrophysiological properties to propafenone [1]. Based on its cellular electrophysiological effects, nicainoprol appears to belong to the I_B class of antiarrhythmic drugs, such as lidocaine and its congeners, by the criteria of Singh and Vaughan-Williams [3] and Anderson et al. [4]. In addition, nicainoprol has such a unique characteristic as occupying a β-blocking property and being devoid of negative inotropic action [1].

To achieve optimal therapeutic benefit from any antiarrhythmic agent,

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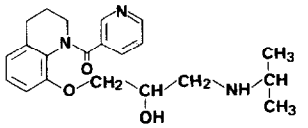
including nicainoprol, it is necessary to understand its disposition characteristics in humans. Furthermore, the pharmacokinetic and pharmacodynamic profiles of such drugs should be clarified, with special emphasis on evaluating the relationships between dose, route, plasma concentration and urinary excretion, prior to the treatment of patients with arrhythmias [4, 5]. It is thus essential to establish an assay method for the drug under study.

There has been no report of an assay method for nicainoprol. We therefore intend to develop the analysis of this new antiarrhythmic agent in biological fluids by high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Chemicals and reagents

Nicainoprol and *p*-chlorodisopyramide as an internal standard (Fig. 1) were supplied by Roussel (Tokyo, Japan). Acetonitrile was of HPLC grade, and dichloromethane, acetic acid, sodium acetate, sodium hydroxide and hydrochloric acid were of reagent grade. All these chemicals were purchased from Wako (Osaka, Japan). Standard stock solutions containing nicainoprol (100 μg/ml) and *p*-chlorodisopyramide (40 μg/ml) were prepared in 0.01 *M* hydrochloric acid. All calibration curves were obtained from spiked plasma or urine samples for the desired concentrations. All stock solutions, and plasma and urine for the calibration curves, were stored at 4°C.



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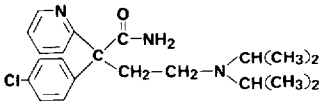

B

Fig. 1. Chemical structures of (A) nicainoprol and (B) *p*-chlorodisopyramide, the internal standard.

Instrumentation and chromatographic conditions

The HPLC analysis was performed by an Altex pump, Model 110A (Altex Scientific, Berkeley, CA, U.S.A.) equipped with a Waters Autosampler system, WISP 710B (Waters Assoc., Milford, MA, U.S.A.), a UVILOG 5IV variable-wavelength UV detector (Oyobunko Kiki, Tokyo, Japan), and Shimadzu C-R1B Chromatopac (Shimadzu, Tokyo, Japan). The reversed-phase HPLC separation was carried out with a UNISIL Q CN column (250 mm × 4.6 mm I.D.), 5 μm particle size (Gaskuro Kogyo, Tokyo, Japan) and the UV wavelength was set at 250 nm with a sensitivity setting at 0.01—0.08 a.u.f.s. The mobile phase consisted of 0.05 *M* sodium acetate buffer (pH 4.0) mixed with glacial acetic acid and acetonitrile (93:7), and was supplied at a flow-rate of 1.5 ml/min at ambient temperature.

Sample preparation

Plasma (1—2 ml) or urine (0.2—1 ml) in a 10-ml PTFE-lined screw-capped tube was mixed with 30 μl of the internal standard solution (40 μg/ml) for plasma analysis or 200 μl for urinary analysis, 0.5 ml of 1 *M* sodium hydroxide and 3 ml of dichloromethane. The tube was capped and shaken by hand for 30 s to avoid micelle formation in plasma, followed by vortex-mixing for 60 s. After centrifugation at 1500 *g* for 10 min, the upper aqueous layer was carefully discarded by aspiration using a Pasteur pipette. Then dichloromethane was transferred into a small glass tube and evaporated to dryness with a gentle air stream at 40°C. The residue was reconstituted with 100– 200 μl of 0.01 *M* acetic acid, and 50—70 μl of this solution were injected onto the chromatograph through the autosampler. Results were calculated from the peak-height ratios. The calibration curves were obtained daily from spiked plasma and urine samples.

Clinical pharmacokinetic study

A healthy male volunteer, 38 years old, participated in the preliminary pharmacokinetic study of nicainoprol after giving a written informed consent. A single 200-mg dose of nicainoprol was orally administered after an overnight fast. Multiple venous blood samples were drawn over the following 48 h, and urine samples were collected at 0—2, 2—4, 4–8, 8–12, 12—24 and 24—48 h after dosing. Blood samples were immediately centrifuged and the plasma samples were separated. Plasma and urine samples were stored at −20°C until analysed.

RESULTS

Representative chromatograms obtained from the drug-free plasma and urine and their standards, together with those samples from a human volunteer who received an oral dose of nicainoprol (200 mg), are shown in Fig. 2. Although a few unknown peaks were derived from almost all plasma extracts, the compounds were well separated and were not affected by those unknowns. Retention times for nicainoprol and the internal standard, *p*-chlorodisopyramide, were 6.3 and 8.6 min, respectively.

The absolute extraction recoveries of nicainoprol and the internal standard from plasma and urine were assessed by comparing the peak heights obtained from the standard stock solutions of the compounds to the drug-free plasma or urine spiked with the respective drug. As can be seen in Table I, the extraction recoveries from plasma averaged 94.8 and 97.0% for nicainoprol at concentrations of 50 and 500 ng/ml, respectively, and 98.9% for the internal standard at 1200 ng/ml. The recoveries from urine were fairly similar to those from plasma (Table I).

The calibration curves were obtained by plotting the peak-height ratios of nicainoprol to the internal standard with different concentrations for plasma and urine samples expected from a nicainoprol dose size (200 mg orally) for the antiarrhythmic therapy. The regression lines were linear over the concentration ranges examined (25—2000 ng/ml for plasma and 0.5—20 μg/ml for

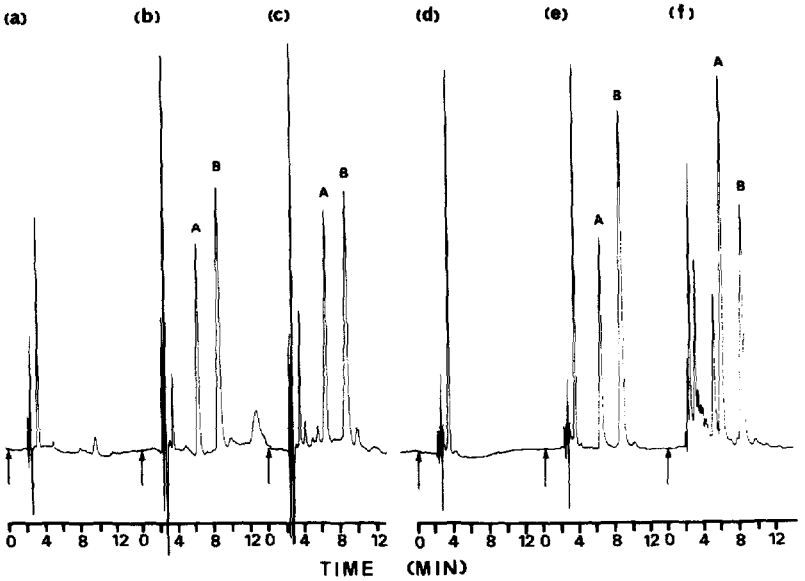

Fig. 2. Chromatograms of extracts from: (a) drug-free plasma; (b) plasma standard containing 500 ng/ml nicainoprol and 1200 ng/ml *p*-chlorodisopyramide (internal standard); (c) plasma of a healthy subject who received 200 mg of nicainoprol orally; (d) drug-free urine; (e) urine standard containing 2 μg/ml nicainoprol and 8 μg/ml internal standard; (f) 12—24-h urine sample of the same healthy subject as noted in (c). Peaks: A = nicainoprol; B = *p*-chlorodisopyramide, the internal standard.

TABLE I

EXTRACTION RECOVERIES OF NICAINOPROL (N) AND THE INTERNAL STANDARD (I.S.) FROM PLASMA AND URINE

Drugs	Concentration	*n*	Recovery (mean ± S.D.) (%)	C.V. (%)
Plasma				
N	50 ng/ml	5	94.8 ± 3.8	4.0
	500 ng/ml	5	97.0 ± 2.6	2.7
I.S.	1200 ng/ml	4	98.9 ± 3.2	3.2
Urine				
N	1 μg/ml	5	94.3 ± 1.2	1.3
	10 μg/ml	5	98.7 ± 2.3	2.3
I.S.	8 μg/ml	4	98.4 ± 0.9	0.9

urine). The correlation coefficients of the calibration curves for both plasma and urine analyses ranged between 0.9979 and 0.9999.

To assess the precision of this analytical procedure, reproducibilities for both within-day and day-to-day variations were determined (Table II). The coefficients of variation (C.V.) for seven different concentrations in the within-day study varied between 1.4 and 9.3% for plasma and between 0.6 and 5.2% for urine samples; those in the day-to-day study ranged from 3.6 to 7.9% for plasma and 2.6 to 6.8% for urine samples. At the same time, the

TABLE II

ANALYTICAL PRECISION AND ACCURACY IN THE DETERMINATION OF NICAINOPROL FROM SPIKED PLASMA AND URINE SAMPLES

Plasma				Urine			
Concentration given (ng/ml)	Concentration observed (mean ± S.D.) (ng/ml)	C.V. (%)	Relative error (%)	Concentration given (μg/ml)	Concentration observed (mean ± S.D.) (μg/ml)	C.V. (%)	Relative error (%)
Within-day variation (n = 5)							
25	26.9 ± 2.24	8.3	7.6	0.5	0.48 ± 0.025	5.2	−4
50	54.2 ± 5.04	9.3	8.4	1	0.95 ± 0.046	4.8	−5
100	108 ± 8.0	7.4	8.0	2	1.96 ± 0.021	1.1	−2
200	197 ± 9.3	4.7	−1.5	5	5.0 ± 0.129	2.6	0
500	509 ± 10.5	2.1	1.8	10	10.1 ± 0.160	1.6	1
1000	968 ± 46.9	4.8	−3.2	20	20.0 ± 0.125	0.6	0
2000	2013 ± 28.2	1.4	0.7				
Day-to-day variation (n = 6)							
50	54.5 ± 4.31	7.9	9.0	1	0.95 ± 0.065	6.8	−5
500	518 ± 18.9	3.6	3.6	10	10.0 ± 0.262	2.6	0

accuracy was evaluated by comparing the given amounts of the drug with those estimated. The observed concentrations were in a good agreement with the actual concentrations: the relative error ranged from −1.5 to 9.0% for plasma, and from −5.0 to 1.0% for urine samples (Table II).

The detection limit was determined by using the diluted working solutions. Nicainoprol can be detected in a concentration as low as 15 ng/ml at a detector attenuation of 0.005 a.u.f.s. (signal-to-noise ratio of 5:1) in plasma with 1 ml of the sample.

The stability of nicainoprol in plasma and urine stored at 4°C was evaluated. Spiked plasma and urine stored at 4°C for two weeks yielded the following average values: 99.0 and 99.6% of the peak-height of nicainoprol when freshly prepared at the concentrations of 500 ng/ml of plasma and 10 μg/ml of urine, respectively. Those average values after storage under the same conditions for 30 and 60 days were 101 and 99% and 98 and 98%, respectively. Similarly, the stability of nicainoprol after the extraction was assessed. The drug reconstituted in 0.01 *M* acetic acid was fairly stable (97 and 98%) at the con-

TABLE III

RETENTION TIMES OF OTHER ANTIARRHYTHMICS AND THEIR ACTIVE METABOLITES

Drug	Retention time (min)	Drug	Retention time (min)
Atenolol	2.9	Mono-N-dealkyldisopyramide (MND)*	4.3
Procainamide	3.1	Disopyramide	5.3
N-Acetylprocainamide (NAPA)*	3.3	Mexiletine	5.4
Monoethylglycinexylidide (MEGX)*	3.6	Quinidine	8.6
Lidocaine	3.9	Diltiazem	10.1
Tocainide	4.1	Propranolol	12.4
Metoprolol	4.3	Verapamil**	—

*NAPA, MEGX and MND are pharmacologically active metabolites of procainamide, lidocaine and disopyramide, respectively.

**Verapamil was not eluted within 20 min under the conditions described.

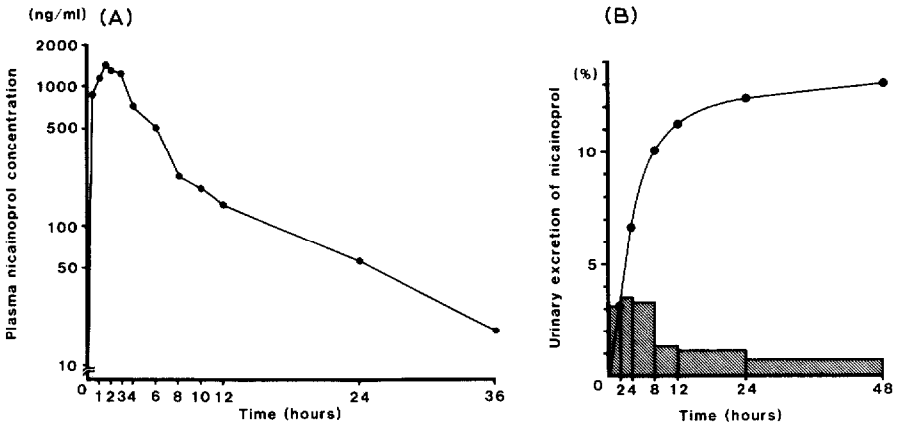

Fig. 3. Plasma concentration—time curve (A) and urinary excretion—time profile (B) of nicainoprol following an oral dose of 200 mg of nicainoprol to a healthy subject. The solid line indicates cumulative urinary excretion and the shaded area represents the fractional amount of urinary excretion in (B). See Table IV for the derived pharmacokinetic parameters for nicainoprol.

TABLE IV

PHARMACOKINETIC PARAMETERS DERIVED FROM A HEALTHY SUBJECT RECEIVING A SINGLE ORAL ADMINISTRATION OF 200 mg OF NICAINOPROL

Abbreviations are: K_a = absorption rate constant; C_{max} = maximum plasma concentration; t_{max} = time to reach C_{max}; $t_{1/2\alpha}$ = distribution phase half-life; $t_{1/2\beta}$ = elimination phase half-life; $[AUC]_0^\infty$ = area under the plasma concentration—time curve from zero to infinity; Cl_p/F = plasma clearance realtive to the extent of availability (F), calculated as Cl_p/F = dose/$[AUC]_0^\infty$; $V_{d(area)}/F$ = apparent volume of distribution relative to the extent of F calculated by the area method as $V_{d(area)}/F$ = dose/β · $[AUC]_0^\infty$; free$[X_u]_0^\infty$ = projected cumulative urinary excretion of free nicainoprol from zero to infinity; Cl_R = renal clearance calculated as free$[X_u]_0^\infty/[AUC]_0^\infty$.

Parameter	Value	Parameter	Value
K_a (h^{-1})	0.828	Cl_p/F (ml/min/kg)	5.1
C_{max} (ng/ml)	1364	$V_{d(area)}/F$ (l/kg)	4.2
t_{max} (h)	1.5	free$[X_u]_0^\infty$ (mg)	26.8
$t_{1/2\alpha}$ (h)	1.33	free$[X_u]_0^\infty$ (% dose)	13.4
$t_{1/2\beta}$ (h)	9.53	Cl_R (ml/min/kg)	0.69
$[AUC]_0^\infty$ (ng/ml · h)	8439		

centrations tested above for both samples for seven days under the same conditions of storage at 4°C.

The possible interference(s) from several other antiarrhythmic agents (which might be concurrently administered with nicainoprol), including some of their active metabolites, which could be extracted under the conditions described, was tested and the data are summarized in Table III. None of the tested drugs (except quinidine, for which the retention time of 8.6 min was identical with that of the internal standard peak) was found to interfere with the analysis of nicainoprol.

The preliminary data on the clinical applicability of the proposed HPLC

method for the nicainoprol pharmacokinetics are demonstrated in Fig. 3, which shows the plasma concentration—time and urinary excretion—time profiles. The derived pharmacokinetic data are listed in Table IV.

DISCUSSION

During the development of the assay method, various extraction and chromatographic conditions were evaluated to determine the optimal conditions for the HPLC analysis of nicainoprol. The extraction solvent, the composition and pH of the mobile phase, and the detection wavelength were carefully assessed, and all these factors were evaluated after the optimal assay conditions had been achieved in accordance with standard practice [6].

Extraction of the urine samples for nicainoprol with dichloromethane brought about much cleaner extracts than with solvents such as diethyl ether and ethyl acetate, though the extraction recoveries were similar. We therefore chose dichloromethane rather than other organic solvent(s). A mobile phase consisting of 0.05 *M* acetate buffer—acetonitrile (93:7) was found to provide a good separation of nicainoprol and the internal standard. Acetate buffer (pH 4.0) produced no interferences from unknown or probably endogenous plasma extracts, and the time required for chromatography could be shortened. Although some small endogenous unknown peaks, which we have not yet excluded, appeared in the chromatograms from almost all plasma extracts (Fig. 2), this situation could easily be avoided by careful timing of the sample injection. Since nicainoprol is not fluorescent, UV detection was considered necessary and the wavelength selected was 250 nm.

We chose an internal standard method to correct possible error(s) in handling pipettes and syringes, and erratic extraction efficiency. Of many compounds tested, *p*-chlorodisopyramide was adopted as the most suitable. The compound, which is non-drug (not clinically used), but is extracted well under the condition used, absorbs UV radiation and is adequately separated from endogenous background peaks from plasma and from the peak of nicainoprol under the chromatographic conditions employed.

The extraction recoveries were sufficient for the analysis of both compounds (Table I). Moreover, the extraction procedures evaluated by precision and accuracy for both within-day and day-to-day analyses were fairly reproducible and reliable (Table II). Excellent linearity was observed for the calibration curves for plasma and urine samples ($r > 0.9979$). From the stability study of nicainoprol in plasma and urine, the drug remained stable if the samples were stored at 4°C for two months and reconstituted in 0.01 *M* acetic acid after extraction for seven days between analyses. Furthermore, other antiarrhythmic drugs (except for quinidine), which may be coadministered with nicainoprol, and some pharmacologically active metabolites did not interfere with the present assay.

To our knowledge, no report is at present available on the pharmacokinetic profile of nicainoprol. Our preliminary results obtained from a healthy person receiving a single oral dose of 200 mg of nicainoprol (Fig. 3) indicate that the plasma concentration—time data can be fitted to a two-compartment model with first-order absorption and elimination. The corresponding pharmaco-

kinetic parameters were calculated by the MULTI program [7]. Based on the data of cumulative urinary excretion of free nicainoprol ($[X_u]_0^\infty$ = 13%, Fig. 3B and Table IV), the drug is assumed to be extensively metabolized. The expected metabolite(s) of nicainoprol could have a shorter retention time than that of the parent drug in a reversed-phase HPLC mode. Indeed, a peak that we assume to be derived from the possible metabolite(s) eluted at 5.6 min, just before that of nicainoprol in the chromatograms of both plasma and urine samples from the healthy subject (Fig. 2c and f). Since no peak was found at the retention time of 5.6 min on the chromatograms of drug-free plasma and urine samples, the peak would be attributable to a metabolite of nicainoprol. However, there has been no information on the metabolites identified and, therefore, the chemical structure(s) is unknown. Based on the chemical structure of the parent compound, nicainoprol (Fig. 1A), one might assume that the N-dealkylated metabolite would be likely to be formed. It would be interesting and important to know if the N-dealkylated metabolite can be measured by the present assay procedure, but this is impossible at present for the reasons given above.

By using the assay method reported here, a study on the pharmacokinetics and pharmacodynamics of nicainoprol in humans is underway in this laboratory, and the results will be reported elsewhere.

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