Determination of Barnidipine in Human Serum and Dog Plasma by HPLC with Electrochemical Detection

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Barnidipine is a 1,4-dihydropyridine calcium antagonist. HPLC was conducted on a polybutadiene coated alumina column using an alkaline mobile phase and an electrochemical detector to determine the content of this drug in serum and plasma. A good linear relationship between barnidipine concentration and peak height was found in 5—500 ng/ml with a correlation coefficient of 0.998. The detection limit was 1 ng/ml. The within-day and day-to-day variations were examined for control human serum. Relative standard deviation of within-day assay for serum spiked with 10 ng/ml barnidipine·HCl was 6.9% and the recovery was 104%. A pharmokinetic study was made in which the time course of barnidipine in dog plasma was followed.

Key words barnidipine; 1,4-dihydropyridine calcium antagonist; HPLC; electrochemical detection; pharmokinetic study.

Barnidipine·HCl, 3-(1-benzyl-2,3,4,5-tetrahydropyrrrole) methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate monohydrorochloride, is a calcium channel blocking antihypertension drug. The determination of drugs of this type is usually conducted by HPLC with UV detection, but in some cases tedious sample pretreatment is required to prevent interference from other substances. HPLC with electrochemical detection is presently being used to determine analogous drugs such as nifedipine and nicardipine in serum or plasma. This method offers specific selectivity with high sensitivity.

Redox reaction pathways of barnidipine were previously clarified by cyclic voltammetry with a glassy carbon electrode and a well-defined anodic peak was shown due to the oxidation of the dihydropyridine moiety to the corresponding pyridine form. Peak height at high pH was proportional to barnidipine concentration without any effect on adsorption. This study was conducted to determine barnidipine in serum by HPLC on a polybutadiene coated alumina column using an alkaline eluent and electrochemical detector. Pharmokinetic observation of barnidipine in dog plasma was also made by this method.

MATERIALS AND METHODS

Materials and Reagents Barnidipine·HCl and the internal standard (I.S.), YC-204 [2-(N-benzyl-N-methylamino) ethylmethyl 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate hydrochloride] were generously provided by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). Stock solutions of barnidipine·HCl and the I.S. were prepared by dissolving them in acetonitrile.

Drugs of chlorpromazine, vitamin C (Wako Pure Chemical Industries, Osaka), acetazolamide, hydrochlorothiazide, amitriptyline, imipramine (Sigma Chemical Company, St. Louis, MO), methyl-t-dopa, clonidine, phenoxycbenzamine (Tokyo Kasel Kogyo Co., Ltd., Tokyo), propranolol, allopurinol and cyproheptadine (Nalacai Tesque Inc., Kyoto) were guaranteed or analytical grade commercial products. Acetonitrile of HPLC grade and other chemicals of reagent grade were obtained from Wako Pure Chemical Industries. Benzene (Wako Pure Chemical Industries) was used following distillation. Deionized water (≥17 MΩ) prepared by a Nano pure purification system (Barnstead Co., Boston, MA, U.S.A.) was used in all experiments. Control human serum, Seraclear-N, was purchased from Nihon shoji Co. (Osaka).

Chromatography The apparatus for HPLC consisted of a constant flow pump (JASCO PU-980, Tokyo), injection valve with a 5 μl loop (Model Rheodyne 7125, Cotati, CA, U.S.A.), an analytical column, Aluspher RP-select B (5 μm, 250 × 4 mm i.d., Merck, Darmstadt, Germany), electrochemical detector with glassy carbon working electrode (Model 840-EC, JASCO, Tokyo) and a recorder (Model LR 4110, Yokogawa, Ltd., Tokyo, Japan). The mobile phase was 0.1 M Britton-Robinson buffer (pH 11.8)-acetonitrile (3:2, v/v), administered at a constant flow rate of 0.9 ml/min. The potential of the detector was set at 0.6 V against an Ag/AgCl reference electrode.

Extraction from Serum or Plasma Into a glass test tube covered on the outside with aluminum foil, 0.5 ml human serum or dog plasma, 0.5 ml of 0.5 M phosphate buffer (pH 11) and 3 ml benzene were pipetted. The tube was shaken mechanically for 5 min and centrifuged at 3000 rpm for 10 min. Then, 2.7 ml of the clear organic layer in the tube was transferred to another test tube containing 50 ng I.S. and evaporated to dryness. The residue was dissolved in 20 ml 0.1 M Britton-Robinson buffer (pH 11.8)-acetonitrile (3:2) and a 5 μl portion was injected into the column.

Pharmokinetic Study Three male beagle dogs weighing 10—12 kg were fasted overnight. On the morning of the following day, each was administered with 5 mg/kg barnidipine·HCl orally (capsule) with 20 ml water. Thirty blood samples (each 6 ml) were obtained from the dogs via the cephalic vein over a period of 8 h (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 h). The samples were centrifuged at 3000 rpm for 10 min in test tubes covered

on the outside with aluminum foil and the plasma was separated and frozen at $-25^\circ C$ until use.

RESULTS AND DISCUSSION

**Electrochemical Detection of Barnidipine in HPLC** The hydrodynamic voltammogram obtained with $1.6 \times 10^{-6}$ m barnidipine from 0.1 to 0.7 V is shown in Fig. 1, along with that of I.S. Barnidipine and I.S. each possesses a dihydropyridine moiety which is oxidized through loss of two electrons and two protons to give the corresponding pyridine. As can be seen in the figure, both compounds underwent oxidation above 0.4 V. Current maxima were attained at 0.6 V. The detection potential for analysis of barnidipine·HCl was thus usually maintained at 0.6 V. Five microliters of solution mixture of barnidipine·HCl and I.S. were injected into the column followed by chromatography in the mobile phase. The retention times of barnidipine and I.S. were 8.5 and 7.3 min, respectively.

Determination was made of barnidipine in a series of solutions containing I.S. Peak height ratio was plotted against barnidipine concentration. A good linear relationship was found at $3 \times 10^{-8}$ to $5 \times 10^{-5}$ m (10 ng/ml to 30 µg/ml) with a correlation coefficient of 0.998. For the $1.0 \times 10^{-6}$ m barnidipine solution, the coefficient of variation was 2.5 ($n=15$).

**Photostability of Barnidipine** In consideration of the light sensitivity of 1,4-dihydropyridine, assessment was made of the photostability of barnidipine. Five milliliters of $2.0 \times 10^{-5}$ m barnidipine in acetonitrile were introduced into each of a number of quartz glass cells, followed by exposure to light from a fluorescent lamp (40 W, laboratory lighting conditions), sunlight and light at 360 nm. The photodegradation of barnidipine is shown in Fig. 2. Although the degradation was least by exposure to light from the fluorescent lamp, there was still a reduction of 10% from the initial concentration after 5 h. Thus, all experiments on this drug should be conducted under shaded conditions.

**Determination of Barnidipine in Serum** The method was then used to determine barnidipine in the control human serum. Figure 3 shows typical chromatograms for extracted sample solutions from the control serum (a) and the serum spiked with 150 ng/ml barnidipine·HCl (b). The peaks of barnidipine and I.S. can be clearly seen in Fig. 3b. Although there was no appreciable interference from endogenous components in serum, peak heights could be obtained by correction for the current of control serum alone.

Barnidipine·HCl at 5—500 ng/ml showed a good linear relationship with the peak height ratio of barnidipine to I.S. with a correlation coefficient of 0.998 (Fig. 4). The detection limit was 1 ng/ml. Precision and recovery were assessed from results of within-day and day-to-day assays for control human serum sample spiked with various amounts of barnidipine·HCl (Table 1). Relative standard deviation within a day and from day to day was less than 10%.

Effects of coadministered drugs such as other antihypertension drugs were also examined by HPLC with electrochemical detection at 0.6 V. The following drugs...
had virtually no effect on barnidipine determination in serum: acetazolamide, methyl-L-dopa, hydrochlorothiazide, clonidine, vitamin C, propranolol, allopurinol, phenoxybenzamine, cyproheptadine, amitriptyline, imipramine and chlorpromazine.

**Pharmacokinetic Study of Barnidipine** Typical chromatograms for extracted sample solutions from dog plasma (a) and the plasma spiked with 50 ng/ml of barnidipine·HCl (b) are shown in Fig. 5. Precision and recovery in the determination of barnidipine in plasma were evaluated based on analysis of blank plasma samples spiked with barnidipine·HCl at 5, 10, 50, or 200 ng/ml (Table 2). The time course of mean barnidipine plasma concentration in three dogs orally administered 5 mg/kg barnidipine·HCl is shown in Fig. 6. Pharmacokinetic data obtained by analysis using a one-compartment model with a first order absorption and elimination rate constant are shown in Table 3. The data demonstrate that barnidipine can be determined in bioavailability studies by this method.

Nifedipine and nicardipine, the most clinically used 1,4-dihydropyridine calcium antagonists, were determined in serum or plasma by HPLC, on a normal phase and a reversed phase column. An electrochemical detector, a sensitive and specific means for detecting 1,4-dihydropyridine derivatives, is ideal for conducting reversed phase HPLC. Many researchers have used silica gel coated with hydrocarbon such as octadecyl silica (ODS) on which mobile phases can be used at 2—8 pH. Alkaline eluents are often suitable for the HPLC separation of basic drugs like barnidipine; in fact, the retention time of barnidipine was found in this study to be quite long, using an ODS column with any mobile phase at pH 2—8. The separation and determination of this drug at pH 11.8 can
Table 3. Pharmacokinetic Data for Barnidipine in Dog Plasma Following Its Oral Administration\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± S.D., ( n = 3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{aw} ) h(^{-1} )</td>
<td>4.86 ± 2.56</td>
</tr>
<tr>
<td>( k_e ) h(^{-1} )</td>
<td>0.353 ± 0.064</td>
</tr>
<tr>
<td>( V_{d} ) l/kg</td>
<td>40.2 ± 3.6</td>
</tr>
<tr>
<td>( AUC_\text{a} ) h·ng/ml</td>
<td>374 ± 45</td>
</tr>
<tr>
<td>( T_{\text{max}} ) h</td>
<td>0.687 ± 0.358</td>
</tr>
<tr>
<td>( C_{\text{max}} ) ng/ml</td>
<td>103.5 ± 9.8</td>
</tr>
<tr>
<td>( CL ) l/h/kg</td>
<td>13.5 ± 1.7</td>
</tr>
</tbody>
</table>

\( a\) Dose of barnidipine·HCl was 5mg/kg. \( b\) \( k_{aw}\) = adsorption rate constant. \( c\) \( k_e\) = elimination rate constant. \( d\) \( V_d\) = volume of distribution. \( e\) \( AUC\) = area under curve. \( f\) \( T_{\text{max}}\) = time required to attain maximum concentration. \( g\) \( C_{\text{max}}\) = maximum concentration. \( h\) \( CL\) = oral clearance.

be conducted within a moderately short time, since a polybutadiene coated alumina column is stable under such an alkaline condition.

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REFERENCES