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Simultaneous Microdetermination of Biperiden, Haloperidol, and Trihexyphenidyl in Plasma and Its Application to Pharmacokinetic Analysis after Concomitant Intravenous Administration of the Drugs to Rabbits

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A gas chromatographic method, using a moving precolumn sample injector and a nitrogen–phosphorus detector, is presented for the simultaneous quantitative analysis of biperiden, haloperidol and trihexyphenidyl in plasma. The drugs were extracted from 1 ml of plasma at pH 10.0 with ether, back-extracted into an acidic solution, and reextracted with ether after alkalinization. Diazepam was used as the internal reference standard.

Processed standard curves of biperiden, haloperidol, and trihexyphenidyl plasma samples were linear over the concentration range of 1.25—50 ng/ml. Recoveries were found to be constant at various spiked doses, and the coefficients of variation were less than 11% for each drug.

Plasma concentration–time courses after simultaneous administration of the three drugs to rabbits were analyzed by model-independent moment analysis. The volumes of distribution at the steady state per body weight of biperiden, haloperidol, and trihexyphenidyl were determined to be 18.5, 16.7, and 17.6 l/kg, respectively. Plasma clearances of the three drugs were 89.7, 61.7, and 79.9 ml/min/kg, respectively.

Keywords——biperiden; haloperidol; trihexyphenidyl; simultaneous microdetermination; gas chromatography; plasma; pharmacokinetics; rabbit; concomitant administration; moment analysis

Introduction

Biperiden (BP) is an anticholinergic drug used for the treatment of Parkinson's syndrome.[2] This drug is used widely as an antiparkinsonian drug with concomitant administration of antipsychotic medication, such as haloperidol (HP).[3] Despite the extensive use of BP, its therapeutic and adverse effects both remain somewhat unpredictable; its pharmacokinetics are not well characterized owing to considerable analytical problems and the low doses used.[4] A little information about BP kinetics in normal subjects has been obtained in recent years[5,6] by using a procedure based on the gas chromatographic method for quantitative analysis of BP in human plasma[5] or serum.[6] Although the method developed by Ottoila and Taskinen[5] is sensitive enough for a pharmacokinetic study, little is known yet about the pharmacokinetics of BP in experimental animals with concomitant administration of HP.

In this report, we describe a method for simultaneous microdetermination of BP, HP, and trihexyphenidyl (TP), which is used in the treatment of Parkinson's syndrome as an analogue of BP. By improving the procedure of Bianchetti and Morselli,[7] a method was developed involving double extraction of the drugs. The pharmacokinetics was determined after concomitant intravenous administration of BP, HP, and TP to rabbits. This is the first pharmacokinetic study of BP in an experimental animal.

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Experimental

Materials——BP and HP were used as supplied by Dainippon Pharmaceutical Co., Osaka, Japan. TP and diazepam, which was chosen as the internal reference standard, were kindly supplied by Lederle Japan Ltd., Tokyo, Japan, and Takeda Pharmaceutical Co., Osaka, Japan, respectively. All other chemicals were of reagent grade and were used without further purification.

Apparatus——A Shimadzu gas chromatograph (GC-7A, Shimadzu, Kyoto, Japan) equipped with a nitrogen–phosphorus detector, NPD (FTD-8, Shimadzu, Kyoto, Japan) was used with a flexible fused silica capillary column (ULBON R HR-52, Sinwa Kako, Kyoto, Japan, 25 mm × 0.24 mm i.d.) previously silanized and coated with 2% OV-101.

Samples (20 μl) of extracts in diethyl ether were injected by the moving precolumn sample injection technique with a 2-min hold at room temperature after the injection. The column was operated isothermally for 4 min at 160 °C, and the temperature program was 160—260 °C, 6 °C/min. Helium carrier gas used at a flow rate of 50 ml/min. The NPD rubidium bead source was heated by a 17 A current at an applied potential of 240 V. Gas flow rates were: hydrogen 5 ml/min and air 110 ml/min.

Stock Solution——An internal standard solution of diazepam (40 ng/ml) in *n*-hexane was prepared.

Calibration Curve and Quantitation——Standard solutions of each dose of BP, HP, and TP (1.25—50 ng/ml) in diethyl ether were prepared. An 0.25 ml aliquot of the internal standard solution was added to and mixed with 1 ml of the standard solution. After evaporation of the solvent, 20 μl of diethyl ether was added. The solution was injected into the gas chromatograph. Quantitative analysis of the peak area ratio was performed by an internal standard method.

Extraction Procedure——For each analysis, 1 ml of plasma was used. A 0.25-ml aliquot of the internal standard solution of diazepam was added as an internal standard to each sample of plasma in a siliconized glass centrifuge tube. A 1-ml aliquot of 1 M Na_2CO_3 buffer (pH 10.0) and 5 ml of diethyl ether were also added. The tubes were then stoppered, shaken vigorously for 5 min at room temperature, and centrifuged for 5 min at 2000 rpm.

The ether phase was transferred to another series of test tubes, each containing 2 ml of 1 N HCl. The tubes were shaken for 2 min and centrifuged for 5 min at 2000 rpm. The resulting ether phase was discarded. Then 1 ml of 3 N NaOH was added to the aqueous phase, together with 5 ml of diethyl ether, and the tubes were shaken again and centrifuged as above. The ether phase was transferred to a glass centrifuge tube, and evaporated. The dry residue was dissolved in 20 μl of diethyl ether, and most of the solution was applied to the chromatograph. The same procedure was employed to determine the percent recovery of spiked drugs. A 1-ml sample of drug-free plasma was mixed with 1 ml of the standard solution of each dose of BP, HP, and TP (1.25—50 ng/ml). The solution was treated as described above, but 4 ml of diethyl ether was added as the first solvent for extraction.

Animal Experiments——Adult male albino rabbits weighing 2.1 kg were fasted for 16 h prior to the experiment but had free access to water.

Under light ether anesthesia, the femoral artery was cannulated with polyethylene tubing. BP, HP, and TP were administered to the rabbit *via* the marginal ear vein. For determination of the plasma concentration, blood samples (*ca.* 2 ml) were withdrawn from the femoral artery *via* the cannula at designated intervals after drug administration, and the blood was collected from the femoral artery in heparinized tubes. The plasma was separated by centrifugation for 10 min at 3000 rpm and kept at 4 °C until assayed.

Pharmacokinetic Analysis——The data on plasma concentration-time courses of BP, HP, and TP were analyzed by model independent moment analysis.[8] The initial plasma concentration was estimated by three-compartment analysis as described below. The last determined plasma concentration was extrapolated to infinite time by using the terminal slope of the log plasma concentration-time curve. The area under the plasma concentration *versus* time curve was estimated by means of the trapezoidal rule.

Non-linear Regression Analysis and Computer Simulation——The data were fitted to a three-compartment pharmacokinetic model by using the NONLIN program[9] with the aid of a FACOM-170F digital computer at the Data Processing Center, Kanazawa University.

Results

Analytical Conditions for Gas Chromatography

Chromatograms of plasma samples spiked with BP, HP, and TP revealed no interference by normal plasma constituents (Fig. 1). The peaks of the drugs were well separated, with retention times of 20 min or less. Retention times for TP, BP, diazepam, and HP were 10.9, 11.3, 13.5 and 19.3 min, respectively. Standard calibration curves obtained after the extraction of BP, HP and TP from the plasma are shown in Fig. 2. The processed standard curves of BP, HP, and TP plasma samples were linear over the concentration range of 1.25—50 ng/ml, and

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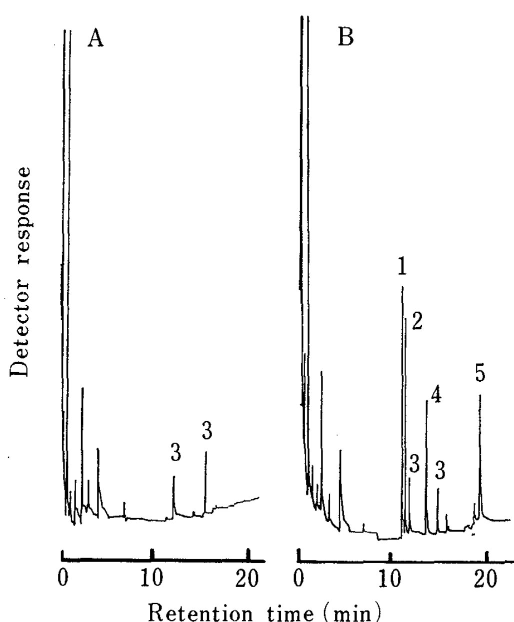

Fig. 1. Chromatograms of Plasma Samples

Detector settings: attenuation 256×. Chromatogram A was obtained with blank plasma. No internal standard was added. Chromatogram B was obtained with blank plasma, spiked with 10 ng/ml each of biperiden, haloperidol and trihexyphenidyl, with diazepam added as an internal standard. Key: (1), trihexyphenidyl; (2), biperiden; (3), unidentified; (4), diazepam; (5), haloperidol.

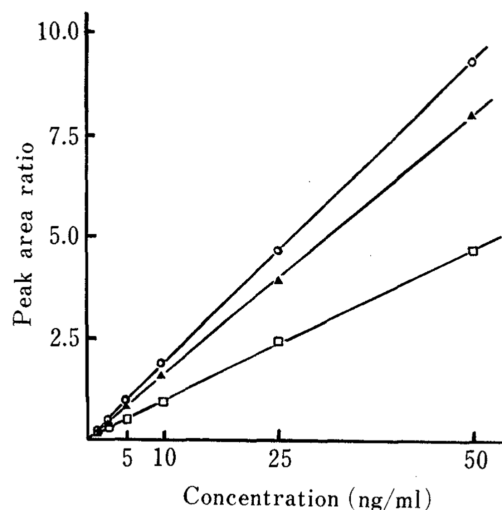

Fig. 2. Calibration Curves Obtained for Biperiden, Haloperidol, and Trihexyphenidyl added to Pooled Rabbit Plasma

The pretreatment procedure and analytical conditions of gas chromatography of biperiden, haloperidol and trihexyphenidyl in plasma samples are described in the text. Key: (▲), biperiden; (□), haloperidol; (○), trihexyphenidyl.

TABLE I. Recovery and Precision of the GC Assay for TP, BP, and HP in Rabbit Plasma

Added (ng/ml)	TP Recovery mean±S.D. (%)	C.V. (%)	BP Recovery mean±S.D. (%)	C.V. (%)	HP Recovery mean±S.D. (%)	C.V. (%)
50.0	86.4±2.3	2.7	84.2±2.9	3.4	83.8±4.6	5.5
25.0	83.6±2.3	2.8	82.8±2.7	3.3	85.6±2.7	3.2
10.0	79.5±3.0	3.8	77.0±3.2	4.2	81.6±4.3	5.3
5.0	80.8±3.6	4.5	82.0±5.4	6.6	84.0±5.4	6.4
2.5	78.8±5.0	6.3	76.8±5.7	7.3	80.0±5.6	7.0
1.25	75.2±6.8	9.0	76.0±5.7	7.5	78.4±8.8	11.2

C.V. = coefficient variation. S.D. = standard deviation.

the assay sensitivities were 0.7, 1.3, and 0.6 ng/ml for BP, HP, and TP, respectively (signal to noise $\simeq 3$). The recovery was estimated by spiking plasma samples with the drugs at various concentrations. Plasma samples were analyzed five times in one run.

The results are shown in Table I together with the coefficients of variation. The recoveries were 79.8, 82.2, and 80.7% for BP, HP, and TP, respectively. The coefficients of variation were less than 11% for each drug.

Plasma Concentrations after Simultaneous Administration of BP, HP, and TP to Rabbits

The time courses of BP, HP, and TP concentrations in plasma are shown in Figures 3-a, 3-b, and 3-c, respectively. The data were analyzed by model-independent moment analysis.[8] Table II shows the area under the plasma concentration *versus* time curve (*AUC*), mean

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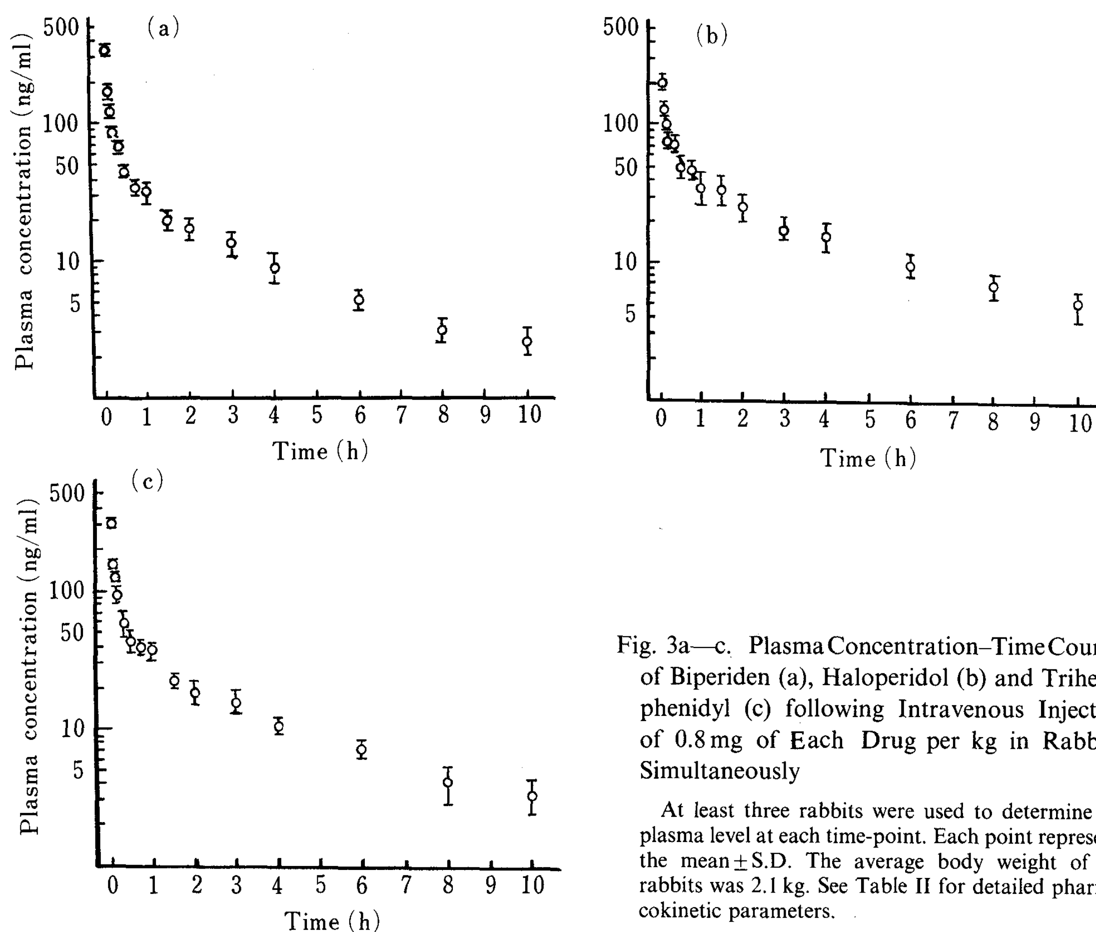

Fig. 3a—c. Plasma Concentration–Time Courses of Biperiden (a), Haloperidol (b) and Trihexyphenidyl (c) following Intravenous Injection of 0.8 mg of Each Drug per kg in Rabbits, Simultaneously

At least three rabbits were used to determine the plasma level at each time-point. Each point represents the mean ± S.D. The average body weight of the rabbits was 2.1 kg. See Table II for detailed pharmacokinetic parameters.

Table II. Pharmacokinetic Parameters of BP, HP, and TP in Rabbits[a]

Parameters	BP	HP	TP
AUC ng min/ml	8.92×10^3	13.0×10^3	10.0×10^3
MRT min	206	270	220
Vd_{ss}/BW l/kg	18.4	16.7	17.6
CL_{tot}/BW ml/min/kg	89.7	61.7	79.7

a) Determined after intravenous bolus injection of 0.8 mg/kg of each drug concomitantly.

residence time (MRT), volume of distribution in the steady state per body weight (Vd_{ss}/BW) and total body clearance per body weight (CL_{tot}/BW) of the three drugs in rabbits. There were no remarkable differences between the parameters of BP and TP. The data were fitted to a three-compartment open pharmacokinetic model by using the NONLIN program.[9] There was fairly good agreement between the calculated and observed concentration *versus* time profiles of the drugs.

Discussion

The most important problem in simultaneous quantitation of drugs by chromatography is to get a fine separation of each peak from interfering factors in the samples without broadening of the peaks. Ottoila and Taskinen[5] described a gas chromatographic assay for BP determination which involves the extraction of BP from serum with hexane. A sensitive gas chromatographic method was reported for quantitative analysis of HP in plasma,[7]

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involving the double extraction of HP from plasma with diethyl ether. To ensure effective sample clean-up, we adopted the method used in HP assay[7] for simultaneous extraction of BP, HP, and TP from plasma, with some modifications. The relative recovery of the drugs was found to be constant. The absolute recoveries of the three drugs, calculated from calibration curves of each drug in standard solutions, were also approximately constant at various sample concentrations (Table I). Although the absolute recovery of HP was similar to the reported value,[7] the recovery of BP was better than that in the method of Ottoila and Taskinen.[5]

Peak broadening of BP and TP was obtained at long sample holding times. In contrast, greater tailing of HP was obtained with short holding times. Therefore, the optimal sample holding time in the precolumn was set at 2 min, with the minimum split ratio of carrier gas flow (purge gas/carrier gas = 3).

As regards the adsorption of nitrogen–containing compounds on silica glass, the column used in this study was reasonable. BP, HP, TP, and the internal standard were eluted, and fully separated within 20 min.

The plasma concentration-time courses in rabbits after intravenous administration of BP, HP, and TP decreased according to a two or more compartment pharmacokinetic model. The values of CL_{tot}/BW and Vd_{ss}/BW, which are important parameters to determine the dosage regimen of the drugs, were determined in a model-independent manner.[8]

The values of Vd_{ss}/BW of these three drugs were in the range of 16.7 to 18.4 l/kg BW, which indicate large tissue distributions. Although the volume of distribution of BP has not yet been estimated after intravenous injection, Holleman *et al.*[6] reported that the total distribution volume of BP in man after oral administration is very great ($V_B/F = 4032$ l). By considering the value of F (*ca.* 0.3),[6] Vd_{ss}/BW of BP can be calculated to be *ca.* 16.7 l/kg BW, which is similar to that of BP in rabbits (Table II). Vd_{ss}/BW of HP was reported to be 17.8 l/kg BW in man after intravenous injection,[10] and this agrees well with the value in rabbits obtained in this study. High plasma clearance was obtained for BP, HP, and TP, as shown in Table II. The plasma clearance of HP was reported to be due to metabolism in rats[11] and man.[10] Since no unchanged BP is excreted *via* the kidney,[12] the total clearance of BP is thought to be high and essentially due to metabolism. Very small residual peaks have been observed in urine by application of our assay procedure (data not shown). Therefore it is reasonable to conclude that there is no interference by metabolites.

To clarify the pharmacokinetics of a drug that has a large distribution volume and a high plasma clearance, a highly sensitive assay method is required. Our assay method has been used successfully for monitoring plasma concentrations of >2 ng/ml of these three drugs simultaneously. Also, the agreement of the pharmacokinetic parameters between man and rabbit suggests that the rabbit is a suitable experimental animal for studies on the pharmacokinetics of BP. A further detailed pharmacokinetic study of BP is proceeding in our laboratory with experimental animals.

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