Effects of Fasting on Biperiden Pharmacokinetics in the Rat

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Abstract □ The effects of fasting on the pharmacokinetics of biperiden in rats were examined. Total clearance of biperiden was >90% ascribable to hepatic clearance and was essentially blood-flow dependent. The number of compartments in the preferred pharmacokinetic model of biperiden changed from three (for normal rats) to two (for fasted rats). The smaller mean residence time (MRT) values found for fasted rats were attributable to decreases in distribution volume. Biperiden showed much higher lipophilicity than haloperidol, thiopental, and hexobarbital, and its tissue-to-plasma partition coefficient in adipose tissue was 20-fold higher than that in muscle. The influence of changes in volumes of adipose tissue and muscle on distribution volume (Vd_{ss}/BW) was evaluated from tissue-to-plasma partition coefficients. The value of Vd_{ss}/BW was predicted to decrease with decrease of adipose tissue, and to increase with decrease of muscle tissue. These results suggest that the observed decrease of Vd_{ss}/BW in fasted rats reflects reduced capacity to trap biperiden in the body, especially in adipose tissue. Possible clinical implications of these results are discussed.

The decrease of body weight that often occurs in diseased individuals is an important physiological alteration influencing tissue distribution of drugs. In order to analyze factors affecting changes of pharmacokinetic parameters arising from decreased body weight, experiments using fasted animals are convenient.

A previous study in our laboratory[1] demonstrated that adipose tissue and muscle account for a considerable part of the distribution volume of biperiden in rabbits. Despite extensive clinical use of biperiden in the treatment of Parkinsonian syndrome,[2] only two pharmacokinetic studies in humans have been reported,[3,4] and interindividual variations of pharmacokinetic behavior are still unclear. Therefore, it is of great interest to investigate interindividual differences in the distribution volume of biperiden in relation to changes of adipose tissue and muscle volumes.

In this work, we investigated the pharmacokinetic behavior of biperiden in normal and fasted rats. The influence of changes of adipose tissue and muscle volumes on the pharmacokinetic parameters of this drug is discussed.

Experimental Section

Materials—Biperiden, haloperidol (Dainippon Pharmaceutical Co., Osaka, Japan, Lot PP 1740 and Lot PN 1376, respectively), and diazepam (Takeda Pharmaceutical Co., Osaka, Japan, Lot OB 164) were used as supplied. All other chemicals were of reagent grade and were used without further purification.

Determination of Apparent Partition Coefficients—The apparent partition coefficients of four drugs, biperiden, haloperidol (belonging to the same therapeutic class as biperiden), and thiopental and hexobarbital (two barbiturates) were determined. Isotonic phosphate buffer, pH 7.4,[5] was used as the aqueous phase. Benzene, chloroform, octanol, or triolein was used as the organic phase. To minimize the volume change due to mutual miscibility, aqueous and organic phases were presaturated with each other. Essentially the same procedure as described previously was used.[6] Briefly, an exactly measured amount (3–100 mL) of each solution was transferred to a siliconized glass-stoppered flask and shaken for 16 h to achieve complete equilibrium at 37 °C. The two phases were separated by allowing the flask to stand for 1 h, and the aqueous phase was centrifuged at 3000 rpm for 10 min. Biperiden in the aqueous phase was extracted into ether and assayed by GC.[7] Apparent partition coefficients at pH 7.4 were calculated by dividing the concentration in the organic phase by the concentration in the aqueous phase without correction for ionization. Concentration of the drugs in the pH 7.4 buffer did not change under the experimental conditions.

Determination of Serum Protein Binding—The extent of binding of biperiden to rat serum protein was measured by the equilibrium dialysis technique with a sample volume of 0.8 mL. Equilibrium concentrations of biperiden in the serum and buffer compartments were reached after 6 h at 37 °C, and were measured at 8 h. Unbound biperiden was calculated as the ratio of the concentrations of the drug in the buffer and serum compartments. In this study, volume shifts between the two compartments were negligible.

Animal Experiments—Male Wistar rats (Sankyo Laboratory Animal Co., Toyama, Japan) were used randomly in this study. They were divided into two experimental groups: normal and fasted. The change of body weight caused by fasting was rapid during the first 4 d, and slower thereafter. Thus, in order to avoid excessive damage, rats fasted for 4 d with free access to water were used in this study. Plasma samples were analyzed for albumin, total protein, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), triglycerides, and nonesterified fatty acid (NEFA), with the aid of an automated Clinalyzer (MS-24, JEOL Co., Tokyo, Japan). Blood analysis was done with a Hemalog D (Corning Co., USA).

Unless otherwise stated, the femoral artery and vein were cannulated with polyethylene tubing (type PE-10; Clay Adams, Becton Dickinson Co., Parsippany, NJ) under light ether anesthesia. Surgery was completed within 30 min. Cannulated rats were kept in a supine position on restraining plates. A 0.24-mL aliquot of the biperiden solution, prepared in saline, was injected over a period of exactly 2 min through the femoral vein cannula. For plasma concentration determination, blood samples (~1–6 mL) were withdrawn from the femoral artery through the cannula at designated time intervals after drug administration and collected in heparinized tubes. Blood was centrifuged at 3000 rpm and plasma was separated. Plasma samples were kept at 4 °C until the time of the assay.

Determination of Hepatic Blood Flow (Q_h)—Hepatic blood flow was estimated using the method of Yokota et al.[8] For this experiment, an additional cannula was placed in the hepatic vein. A priming dose of indocyanine green was injected into the femoral vein, followed immediately by a constant infusion of the dye into the femoral vein through the cannula at a rate of 13–27 μg/min, yielding a steady-state plasma level of 10–20 μg/mL. Two blood samples (0.2 mL each) were simultaneously withdrawn from the hepatic vein and femoral artery every 10 min starting 60 min after initiation of the dye injection. Plasma was separated by centrifugation and stored at 4 °C until assayed. Aliquots (50–100 μL) of plasma were diluted 10- to 20-fold with distilled water and quantified by measurement of absorbance at 800 nm. Hepatic blood flow was calculated using the equation of Bradley et al.[9]

Determination of Hepatic Extraction Ratio (ER)—The procedure used to measure the hepatic extraction ratio of biperiden was essentially the same as that described previously.[10] A biperiden solution in saline was infused into rats through the femoral vein cannula at the rate of 0.765 mL/h (5.32 mg/mL of biperiden) after giving an intravenous bolus injection of a priming dose (3.2 mg/kg biperiden). After 16 h, pseudo-steady-state biperiden plasma concen-

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Table I—Physiological and Biochemical Parameters of Rats[a]

	Weight at Time of Experiment, g	Hct, %	Albumin, g/dL	Hemoglobin, g/dL	NEFA, mEq/L[b]	GOT, IU/L[c]	GPT, IU/L[d]	Hepatic Blood Flow Rate, Q_h, mL/min/kg
Normal rats	290 ± 4 (45)	42.3 ± 2.2 (4)	4.6 ± 0.4 (4)	15.2 ± 0.7 (4)	608 ± 72 (4)	134 ± 17 (4)	49.5 ± 5.4 (4)	59.7 ± 4.0 (6)
Fasted rats	238 ± 4 (36)	48.1 ± 1.1 (4)	4.6 ± 0.2 (4)	17.5 ± 0.3 (4)	978 ± 101 (4)	163 ± 14 (4)	43.8 ± 2.9 (4)	52.4 ± 5.0 (5)
	$p < 0.001$	$p < 0.01$	NS[e]	$p < 0.01$	$p < 0.01$	NS[e]	NS[e]	$p < 0.05$

[a] Each value represents the mean ± SEM; the number of experiments is given in parentheses. [b] Nonesterified fatty acid. [c] Glutamic oxaloacetic transaminase. [d] Glutamic pyruvic transaminase. [e] No significant difference.

trations were achieved, and plasma concentrations of biperiden in the femoral artery and hepatic vein were determined.

Determination of the Tissue-to-Plasma Partition Coefficient (K_p)—To determine the tissue-to-plasma partition coefficients at steady state, a biperiden solution was infused at the rate of 0.765 mL/h (5.32 mg/mL biperiden) after giving an intravenous bolus injection of a priming dose (3.2 mg/kg biperiden) into normal rats. After 16 h, the rats were sacrificed for sampling of adipose and muscle tissues from the lower back. Tissues were quickly excised, rinsed well with ice-cold saline, blotted dry, and weighed. The procedure for obtaining tissue homogenates was essentially the same as described previously.[10]

Determination of Blood-to-Plasma Concentration Ratio (*RBP*)—A conventional in vitro method was used as follows. After administration of heparin at a dose of 0.1 mL/100 g body weight (100 U), whole blood was collected via the femoral artery cannula. Aliquots (0.1 mL) of isotonic buffer solution containing various amounts of biperiden were added to 5 mL of whole blood. Samples were incubated with slow shaking for 30 min at 37 °C. Concentrations of biperiden in plasma (obtained by centrifugation) and in whole blood were then assayed.

Analytical Procedures—Drug concentrations of biperiden and haloperidol in aqueous solution were determined by GC.[7] The concentrations of biperiden in plasma and urine were determined by GC as described in the preceding paper.[7] In order to detect low concentrations of biperiden in plasma, an amount of plasma three times greater than that used in our previous study[7] was used. The detection limit of the method was 0.3 ng/mL. To determine the concentrations of biperiden in tissues, the same method was applied to tissue homogenates. The coefficients of variation of estimated concentration in plasma and tissue homogenate were <10%. Calibration curves were obtained by the same method for each biological sample. The detection limit of the method giving the same precision was 5 ng/mL for homogenized samples. An HPLC assay method was employed to determine concentrations of hexobarbital and thiopental in the aqueous phase after partitioning into the organic layer. A 0.2-mL aliquot of drug solution was mixed with 1 mL of 1 M phosphate buffer (pH 5.0) and then extracted into 5 mL of ethyl acetate containing 2–6 μg/mL of amobarbital as an internal standard. After evaporation of the solvent, the residue was dissolved in 100 μL of methanol, and then 20 μL of the solution was injected into the chromatograph (model ALC/GPC 204, Waters Associates Inc., Milford, MA). Detection was at 230 nm. A reversed-phase packed column (Zorbax ODS, Shimadzu-Dupont) was used. The mobile phase was methanol:0.01 M monobasic sodium phosphate (60:40) at a flow rate of 1.2 mL/min. Retention times for hexobarbital and thiopental were 6.2 and 9.4 min, respectively. Peak heights and/or peak areas were used for calibration curves obtained daily.

Data Analysis—Biperiden data were analyzed by using both model-independent moment analysis[11] and compartment analysis. Area under the plasma concentration to infinite time versus time curve (AUC) was estimated using the trapezoidal rule; AUC was extrapolated to infinite time from the last determined plasma concentration by using the terminal slope of the log plasma concentration–time curve. Mean residence time (MRT), steady-state distribution volume per body weight (Vd_{ss}/BW), and total body clearance per body weight (CL_{tot}/BW) were estimated as described by Yamaoka et al.[11] Data were fitted to a two- or three-compartment pharmacokinetic model using the MULTI program[12] together with the values of Akaike's information criterion (AIC).[13] Sampling schemes were designed to obtain four to eight data points in each exponential phase. During the fitting procedures, each concentration was weighted by the reciprocal of its square.

Results

Effects of Fasting in Rats—The physiological and biochemical data for rats used in the present study are summarized in Table I. Interanimal variabilities of biochemical parameters in fasted rats were comparable to those in normal rats, even though the body weight of the fasted rats was ~18% less than that of normal rats. Values of hepatic blood flow, Q_h, were significantly different in fasted and normal rats. There were no significant differences in GOT and GPT levels.

Lipophilicity of Biperiden—As shown in Table II, biperiden had the highest lipophilicity in all systems tested.

Protein Binding and Blood-to-Plasma Concentration Ratio (*RBP*)—Both the extent of biperiden binding to serum protein and *RBP* were constant over a wide concentration range (25–1000 ng/mL). Values of the serum unbound fraction were 0.110 ± 0.001 (mean ± SEM, n = 16) and 0.179 ± 0.006 (mean ± SEM, n = 16) for normal and fasted rats, respectively, and the difference was statistically significant ($p < 0.001$). Values of *RBP* were 1.16 ± 0.02 (mean ± SEM, n = 12) for normal rats and 1.13 ± 0.05 (mean ± SEM, n = 10) for fasted rats, and there was no significant difference.

Pharmacokinetics of Biperiden in Rats—Time courses of biperiden plasma concentration in normal and fasted rats are shown in Fig. 1. The behavior of biperiden suggests linear pharmacokinetics because of constant protein binding and RBP, and the linear terminal elimination rate after intravenous injection. Iterative nonlinear least-squares analysis of the data in Fig. 1 provided the parameters listed in Table III, together with the AIC values. The minimum AIC value was obtained for a three-compartment model in normal rats, while AIC became minimum for a two-compartment model in the case of fasted rats. The results of model-independent moment analysis are also listed in Table III. There is good agreement of AUC, Vd_{ss}/BW, and CL_{tot}/BW values between compartment-model and model-independent moment analyses. From the infusion study, the values of *ER* were determined to be 0.910 ± 0.037 (mean ± SEM, n = 4) and 0.870 ± 0.054 (mean ± SEM, n = 4) for normal and fasted rats, respectively. The difference was not significant. By using

Table II—Partition Coefficients of the Drugs Tested

Substrate	Partition Coefficient			
	$P_{benzene}$[a]	P_{chl}[b]	P_{oct}[c]	P_{triol}[d]
Biperiden	6020	22064	678	20828
Haloperidol	598	6913	485	10375
Thiopental	52	311	508	133
Hexobarbital	28	233	42	17

[a] Between benzene and pH 7.4 isotonic phosphate buffer. [b] Between chloroform and pH 7.4 isotonic phosphate buffer. [c] Between octanol and pH 7.4 isotonic phosphate buffer. [d] Between triolein and pH 7.4 isotonic phosphate buffer.

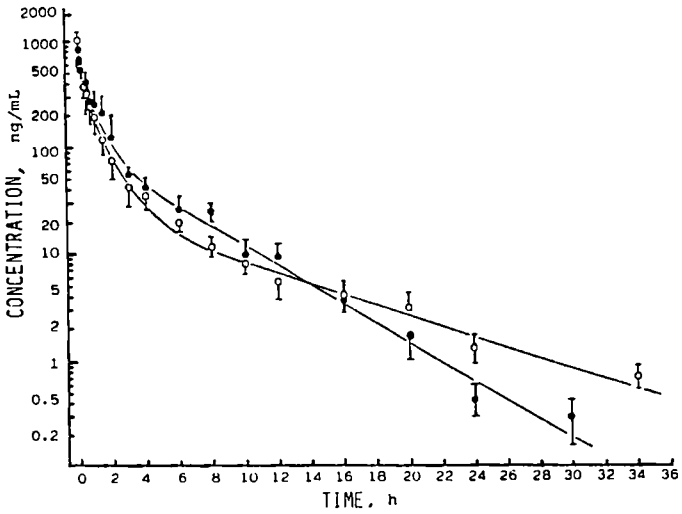

Figure 1—*Plasma concentration–time courses of biperiden in normal (○) and fasted (●) rats following a 3.2-mg/kg bolus iv injection. The lines were fitted by applying the MULTI program (ref. 12) to the equations of three-compartment and two-compartment models for normal and fasted rats, respectively. At least three rats were used at each time point. The average body weights of rats are listed in Table I. Each point represents the mean ± SEM (see Table III for details of pharmacokinetic parameters).*

these values of ER, RBP, and Q_h (Table 1), hepatic clearances of biperiden were calculated to be 62.5 and 51.5 mL/min/kg BW for normal and fasted rats, respectively. These values account for >90% of the values of CL_{tot}/BW which were obtained for both groups of rats from moment analysis (Table III). This finding is consistent with the fact that urinary recovery of intact biperiden was only 1.7 ± 0.2% (mean ± SEM, n = 3) of drug administered intravenously in normal rats.

The K_p values of adipose tissue and muscle at steady state in normal rats were determined to be 58 ± 6 (mean ± SEM, n = 3) and 3 ± 1 (mean ± SEM, n = 3), respectively. Since products of standard anatomical tissue volume (V_t)[10] and K_p were 1.93 ± 0.35 L/kg BW (mean ± SEM, n = 3) and 1.50 ± 0.45 L/kg BW (mean ± SEM, n = 3) for adipose tissue and muscle, respectively, the adipose tissue and muscle accounted for 25% of the value of Vd_{ss}/BW obtained from moment analysis (Table III). Values of the product of the tissue blood flow rate (Q_t)[10] and RBP divided by $V_t \cdot K_p$, as a measure of the distribution rate of drug into each tissue, were 0.02 min^{-1} for muscle and 0.005 min^{-1} for adipose tissue.

Discussion

In the present study, we examined the effect of fasting on the pharmacokinetics of biperiden by comparing physiological and biochemical parameters in normal and fasted rats. As shown in Table I, fasted rats had a markedly decreased body weight, there was small interindividual variation, and there was no change of hepatic function caused by fasting. The results support the idea that a decrease in body weight results in a reduced capacity to trap biperiden in the body.

As can be seen in Table III, despite a larger CL_{tot}/BW value in normal rats than in fasted rats, the MRT value was smaller in the latter. Since urinary recovery of intact biperiden was negligible and values of CL_{tot}/BW in normal and fasted rats were essentially consistent with hepatic clearances, the CL_{tot}/BW can be well explained in terms of hepatic blood flow rate-limited elimination. Therefore, decreased CL_{tot}/BW in fasted rats is considered to be predominantly due to change in the hepatic blood flow rate. As shown in Table I, hepatic blood flow tended to decrease in fasted rats. Because MRT is equal to the ratio of Vd_{ss}/BW and CL_{tot}/BW,[11] the small MRT value of fasted rats should be the result of a decreased distribution volume of biperiden.

In order to analyze factors affecting changes in Vd_{ss}/BW, we derived an equation by means of the physiologically based pharmacokinetic approach.[14] Assuming that fasting influences only volumes of adipose tissue and muscle, the percent change of Vd_{ss}/BW caused by fasting may be written as

Table III—Pharmacokinetic Parameters After Intravenous Administration of Biperiden (3.2 mg/kg) to Rats

Parameter	Normal Rats		Fasted Rats	
AUC, ng·min/mL[a]	47200 ± 2100		59000 ± 3140	
MRT, min[a]	206 ± 13		172 ± 12	
Vd_{ss}/BW, L/kg[a]	14.0 ± 1.4		9.34 ± 1.08	
CL_{tot}/BW, mL/min/kg[a]	67.7 ± 3.0		54.3 ± 2.9	
	Two-Compartment Model	Three-Compartment Model	Two-Compartment Model	Three-Compartment Model
A, ng/mL[b]	631 ± 70	763 ± 255	615 ± 75	623 ± 81
B, ng/mL[b]	31.1 ± 5.3	388 ± 73	95.8 ± 15.8	95.0 ± 63.5
C, ng/mL[b]	—	26.3 ± 3.9	—	3.27 ± 40.7
α, min^{-1}[b]	0.0207 ± 0.0022	0.129 ± 0.0062	0.0213 ± 0.0035	0.0219 ± 0.0042
β, min^{-1}[b]	0.00203 ± 0.00017	0.0146 ± 0.0019	0.00349 ± 0.00016	0.00349 ± 0.00002
γ, min^{-1}[b]	—	0.00189 ± 0.00013	—	0.00269 ± 0.0001
V_1, L/kg[b]	4.83	—	4.50	—
V_2, L/kg[b]	8.92	—	4.80	—
Vd_{ss}/BW, L/kg	13.8	—	9.30	—
AUC, ng·min/mL	45800	46400	56300	56900
CL_{tot}/BW, mL/min/kg	69.9	69.0	56.8	56.3
AIC[b]	1.88	−8.44	2.24	7.24

[a] Determined by model-independent moment analysis (ref 11). [b] Calculated by the MULTI program (ref 12).

$$Vd_{ss}/BW\ \% = (K_{p,o}\cdot V_o + K_{p,i}\cdot\alpha V_{t,i})/BW/(K_{p,o}\cdot V_o + K_{p,i}\cdot V_{t,i})/BW - 1 \times 100 \quad (1)$$

where $V_{t,i}$ is the tissue (i) volume, α represents the degree of decrease of the tissue (i) volume caused by fasting, V_o is the sum of tissue volumes in the body excluding tissue (i), and $K_{p,i}$ and $K_{p,o}$ represent the tissue-to-plasma concentration coefficients for tissue (i) and the other tissues, respectively, of which the latter can be defined as follows:

$$K_{p,o} = (Vd_{ss} - K_{p,i}\cdot V_{t,i})/V_o \quad (2)$$

Taking the observed K_p values of 58 and 3 for adipose and muscle tissues, respectively, and the $K_{p,o}$ value of 12.5 calculated from eq. 2, the effect of fasting on Vd_{ss}/BW can be discussed in terms of eq. 1. The adipose tissue content amounts to ~4% of total body weight in normal rats,[10] so if a 4% decrease of total body weight is assumed to occur solely in adipose tissue, a 13% decrease in the Vd_{ss}/BW of biperiden is predicted. In contrast, if 50% of muscle tissue decreased, a 26% increase, rather than a decrease, in Vd_{ss}/BW is predicted.

Interestingly, the most suitable pharmacokinetic models for biperiden were three- and two-compartment models in normal and fasted rats, respectively (see AIC values in Table III). The terminal elimination phase in normal rats was slower than that in fasted rats. In the two-compartment pharmacokinetic analysis, the V_2:V_1 ratio decreased to about half in fasted rats. Adipose tissue can be considered to belong to a compartment different from the compartment including the plasma, because the value of $(RBP\cdot Q_t)/(K_p\cdot V_t)$ in adipose tissue was extremely small. The decrease in V_2:V_1 may be the result of a decreased volume of adipose tissue. Thus, it seems reasonable to conclude that the decreased values of MRT and Vd_{ss}/BW of biperiden can be mainly attributed to a decrease in adipose tissue volume in fasted rats.

Considering the 1.6-fold change observed in the serum unbound fraction between normal and fasted rats, V_1 and V_2 are expected to be larger in fasted than normal rats, though in fact V_1 was not altered and V_2 decreased. This may suggest that part of the 18% body weight decrease (52 g), which could not be wholly accounted for by adipose tissue (standard adipose tissue volume = 10 mL), reflects a decrease in volume of a slower distribution compartment. Details of this mechanism, together with factors determining the serum protein binding change, remain to be clarified.

In conclusion, biperiden pharmacokinetics are greatly influenced by small changes in the volume of adipose tissue because of the extremely high lipophilicity of the drug. These characteristic properties of biperiden in distribution volume are important since they imply that the clinical dosage regimen of biperiden should be changed depending on changes in the mass of adipose tissue and muscle in individual patients. Changes in adipose and muscle tissue volume may cause big interindividual differences in distribution volume. In particular, we can predict that the opposite effects of changes in adipose tissue and muscle on Vd_{ss}/BW may produce a remarkable increase in Vd_{ss}/BW in aged obese patients in whom there is a simultaneous increase of adipose tissue and decrease of muscle.

References and Notes

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