Comparative Pharmacokinetics of Sulpiride and N-[(1-Butyl-2-pyrrolidinyl)methyl]-2-methyl-5-sulfamoyl-2,3-dihydrobenzofuran-7-carboxamide Hydrochloride, a New Lipophilic Substituted Benzamide in Rats

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The pharmacokinetics of a new lipophilic substituted benzamide *N*-[(1-butyl-2-pyrrolidinyl)methyl]-2-methyl-5-sulfamoyl-2,3-dihydrobenzofuran-7-carboxamide hydrochloride (1) and sulpiride in both plasma and brain were investigated in rats.

The octanol–water partition coefficients of the base of 1(2) and sulpiride were 6.3 and 0.2, respectively. The eliminations of 2 from plasma and brain were similar to those of sulpiride. The systemic bioavailabilities of 1 and sulpiride after oral administration of 200 mg/kg were 60.9 ± 10.9 and 18.2 ± 6.4%, respectively. The brain concentrations of 2 were about 2—3 times higher than those of sulpiride until 4 h after oral administration of 100 mg/kg. The brain/plasma ratios of 2 were about 2 times higher than those of sulpiride. These results indicate that the penetration of 2 through the gastrointestinal membrane and/or the blood-brain barrier are higher than those of sulpiride.

Keywords new lipophilic benzamide; sulpiride; lipophilicity; pharmacokinetics; blood-brain barrier; bioavailability; membrane permeability; rat

Sulpiride, unlike most other neuroleptics, is a selective antagonist of the dopamine D_2 receptors.[1] This drug is a substituted benzamide that has antidepressant, antianxiety and antipsychotic activities.[2] On the other hand, sulpiride has a much lower incidence of extrapyramidal side effects.[3]

The excellent neuroleptic properties of sulpiride require a large amount of clinical dosage due to its low bioavailability as well as low penetration through the blood-brain barrier.[4] Therefore, sulpiride can induce endocrine effects, amenorrhea or galactorrhea, probably as a result of direct interaction with prolactin cells.

Membrane permeability should depend on the oil–water partition coefficient if transfer occurs by simple diffusion through an aporous lipoid membrane.[5] The lipophilic properties of drugs are important factor in drug design. Recently, a new lipophilic substituted benzamide drug, namely the *N*-[(1-butyl-2-pyrrolidinyl)methyl]-2-methyl-5-sulfamoyl-2,3-dihydrobenzofuran-7-carboxamide hydrochloride (**1**) has been introduced as a potential new antipsychotic drug.[6] Administered orally, **1** was 10 times stronger than sulpiride in inhibiting apomorphine (0.5 mg/kg, s.c.)-induced hyperactivity in mice, and administered intravenously or intracerebroventricularly, it was 2 times or one third as strong, respectively.[7] It is presumed that the penetration of the base of **1**(**2**) into the systemic circulation and/or brain is higher than those of sulpiride. In the present study, the systemic bioavailability and the permeability of the blood-brain barrier of **1** and/or **2** were investigated by direct comparison with those of sulpiride in rats.

Experimental

Materials **1**, **2** (the base of **1**), *N*-[(1-propyl-2-pyrrolidinyl)methyl]-2-methyl-5-sulfamoyl-2,3-dihydrobenzofuran-7-carboxamide (**3**) and sulpiride were synthesized in our laboratories. Figure 1 shows their chemical structures. All other chemicals were standard commercial products of analytical grade.

Animals Male Wistar rats were used and were fasted for 24 h before drug treatment. All rats were allowed free access to water but no food was given.

Bioavailability Studies Rats of 275—380 g body weight were used. The jugular vein was cannulated with silicon tubing under light gas anesthesia (halothane : N_2O : O_2) according to the method of Upton.[8] The technique involves cannulating the common carotid artery. Rats were allowed to recover for at least 4 h before the administration of a drug. Compound **1** or sulpiride was orally administered at a dose of 200 mg/kg to animals. Each drug was suspended in 0.5% methylcellulose (MC) solution at a concentration of 20 mg/ml. To determine their bioavailabilities, the drugs were administered intravenously into the jugular vein at a dose of 20 mg/kg. **1** was dissolved in distilled water (10 mg/ml) and sulpiride in 0.5% acetic acid (20 mg/ml).

Blood samples (0.3 ml) were collected from a common carotid artery at designated time intervals after administration. The samples were centrifuged for 15 min at 3000 rpm to obtain plasma (0.1 ml), which was kept frozen at −20 °C until analysis.

Measurements of the Brain/Plasma Ratios Rats of 155—230 g body weight were used. The animals were orally administered with 30 or 100 mg/kg of **1** and 100 or 300 mg/kg of sulpiride. The 0.5% MC suspensions containing 3 or 10 mg/ml of **1** and 10 or 30 mg/ml of sulpiride were used for administration. At designated times after oral administration, each rat was lightly anesthetized with ether and blood was collected from the *vena cava* inferior; the samples (2 ml) were treated as described above. Immediately after blood collection, perfusion of the common carotid artery was begun with normal saline (about 200 ml) at a constant rate (10 ml/min). At the end of the 20 min perfusion, the rat's head was decapitated and stored at −20 °C.

Analytical Methods The plasma concentration of **2** was determined by high-performance liquid chromatography (HPLC). To 0.1 ml of plasma samples were added 100 ng of **3** as an internal standard, 0.1 ml of 0.1 M tris(hydroxymethyl)aminomethane (Tris) and 1.0 ml of an organic mixture of *n*-hexane–dichloroethane (1 : 1, v/v). The mixture was then shaken for 10 min. After centrifugation, 0.8 ml of the organic layer was taken and evaporated at 25 °C. The residue was dissolved in 50 μl of the mobile phase described below and aliquots were used for the analysis. The HPLC system used consisted of a LC-6A, a RF-530 fluorescence detector and a C-R3A integrator recorder (Shimadzu, Kyoto, Japan). The detector was prepared by setting excitation and emission wavelengths at 307 and 358 nm, respectively. Samples were injected *via* a Rheodyne 7125 injector fitted

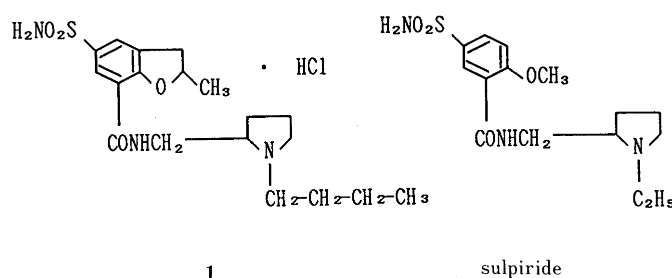

Fig. 1. Chemical Structures of **1** and Sulpiride

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with a 20 μl loop. Separations were performed on a TSK gel ODS 120T (15 cm × 4.6 mm i.d., 5 μm particle size; TOSOH, Tokyo, Japan) with a mobile phase of 0.1 M ammonium acetate–acetonitrile (5 : 2, v/v, adjusted to pH 3.5 with perchloric acid) at an ambient temperature and a flow rate of 1.0 ml/min. The lower limit of the assay of **2** in plasma was 0.05 μg/ml.

Sulpiride concentrations in plasma were also determined using a HPLC method described by Alfredsson *et al.*[9] with a minor change. To 0.1 ml of plasma samples were added 0.1 ml of 0.1 M Tris and 2.0 ml of dichloroethane. The mixture was then shaken for 10 min. After centrifugation, 1.6 ml of the organic layer was taken and evaporated at 25 °C. The residue was dissolved in 50 μl of a mobile phase of 0.05 M ammonium acetate–acetonitrile (85 : 15, v/v) and aliquots were used for the analysis. The HPLC system used was the same as that above. The assay limitation of sulpiride by this method was 0.2 μg/ml.

Brain concentrations of **2** and sulpiride were also determined by the HPLC method. Frozen whole brains were weighed and homogenized in 2 ml of 0.1 M Tris. A portion (1 ml) of homogenate was used for the analysis. The same procedures as for the plasma sample were applied.

Pharmacokinetic Analysis The plasma concentration data for an individual animal after intravenous administration of **1** or sulpiride were fitted to the following equation:

$$C_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}$$

where C_p is the plasma drug concentration at time t, A and B are ordinate axis intercepts, and α and β are the hybrid rate constants, respectively. Pharmacokinetic analysis of oral dosage forms was performed using model independent methods because the plasma concentration data did not fit the compartment model. The peak plasma concentration (C_{max}) and the time taken for attaining the peak concentration (T_{max}) were determined from the individual plasma concentration–time curves. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule and then added to the value of plasma concentration at the detected last time divided by λ (the terminal elimination rate constant), which was calculated by the least squares method with a semi-logarithmic scale.

Total plasma clearance (CL_{total}) was calculated by the following equation:

$$CL_{total} = \text{dose}_{i.v.}/AUC$$

The apparent volume of distribution at steady-state (Vd_{ss}) was estimated by the following equation:

$$Vd_{ss} = \text{dose}_{i.v.} \cdot AUMC/AUC^2$$

where $AUMC$ is the total area under the first moment of the drug concentration curve from zero to infinity.

The brain drug concentration data for an individual animal were fitted to the following equation:

$$C_b = A \cdot e^{-k_2 \cdot t} - B \cdot e^{-k_1 \cdot t}$$

where C_b is the brain concentration at time t, A and B are ordinate axis intercept, and k_1 and k_2 are correspond to the apparent first order permeability rate constant from plasma to brain and the apparent first order elimination rate constant from brain to plasma, respectively.

The systemic bioavailability (BA) was calculated by the following equation:

$$BA = AUC_{p.o.} \cdot \text{dose}_{i.v.}/AUC_{i.v.} \cdot \text{dose}_{p.o.} \times 100$$

Measurements of the Octanol-Water Partition Coefficient The partitionings of sulpiride and **2** between 50 mM Tris–HCl buffer (pH7.2) and *n*-octanol were determined essentially as described by Ritschel.[10] The drug was dissolved in an aqueous solution at a concentration of 1 μg/ml. One hundred ml of buffer and 20 ml of *n*-octanol were placed in an Erlenmeyer flask with a glass stopper and mechanically shaken for 4 h at 37 °C. The concentration of drug in the aqueous phase was determined by the HPLC method as described above. The partition coefficient (K_p) was calculated by the following equation:

$$K_p = (C_1 - C_2) \cdot a / C_2 \cdot b$$

where C_1 and C_2 are the drug concentration in the aqueous phase before and after equilibration, and a and b are the volume of aqueous and organic phase, respectively.

Results

Figure 2 shows the plasma concentration–time curves of **2** and sulpiride after intravenous administration to rats at a dose of 20 mg/kg. The pharmacokinetic parameters of both drugs are listed in Table I. The elimination of **2** was similar to that of sulpiride in rats. However, the CL_{total} and Vd_{ss} of **2** were about 2 times higher than those of sulpiride, respectively.

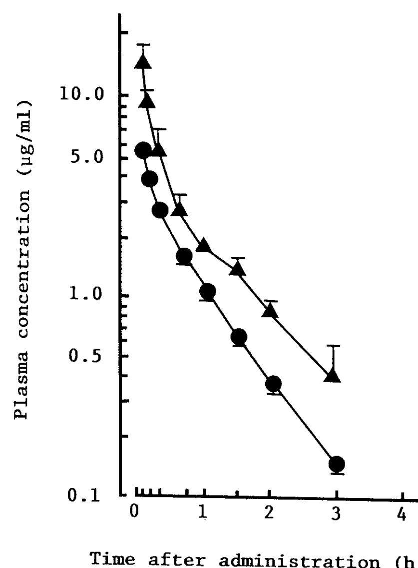

Fig. 2. Plasma Concentration–Time Curves of **2** and Sulpiride after Intravenous Administration

●, **2**; ▲, sulpiride. Each point represents the mean of 4 rats and vertical bar indicates S.D. **1** or sulpiride was administered at a dose of 20 mg/kg.

Table I. Pharmacokinetic Parameters for **2** and Sulpiride in Rats

Parameter	**2**		Sulpiride	
	20 mg/kg (i.v.)	200 mg/kg (*p.o.*)	20 mg/kg (i.v.)	200 mg/kg (*p.o.*)
C_{max} (μg/ml)	—	6.97 ± 0.49	—	3.87 ± 1.47
T_{max} (h)	—	0.45 ± 0.30	—	1.67 ± 1.22
AUC (μg·h/ml)	3.58 ± 0.30	22.38 ± 3.47	8.03 ± 1.40	15.21 ± 5.29
$T_{1/2}$ (h)	0.71 ± 0.03	1.84 ± 0.42	0.94 ± 0.22	1.69 ± 0.36
α (h^{-1})	4.97 ± 1.00	—	6.99 ± 3.00	—
β (h^{-1})	0.982 ± 0.046	—	0.776 ± 0.196	—
Vd_{ss} (l/kg)	5.10 ± 1.30	—	2.11 ± 0.17	—
CL_{total} (l·h/kg)	5.48 ± 0.47	—	2.46 ± 0.45	—

Each value represents the mean ± S.D. of 4 or 5 rats. —: not calculated.

The plasma concentration–time curves following oral administration of the drugs at a dose of 200 mg/kg are shown in Fig. 3. The parameters were calculated and are shown in Table I. The plasma concentrations of **2** were higher than those of sulpiride up to 3 h after the oral administration. The C_{max} and AUC of **2** were about 1.8 and 1.5 times greater than those of sulpiride, respectively. The T_{max} of sulpiride was about 3.6 times longer than that of **2**.

The systemic bioavailabilities of **1** and sulpiride are listed in Table II, where the partition coefficients of **2** (free base of **1**) and sulpiride are also shown. The bioavailability of **1** was about 3 times higher than that of sulpiride.

Figure 4 shows the brain concentration–time curves of **2** and sulpiride after oral administration of **1** (30 or 100 mg/kg) and sulpiride (100 or 300 mg/kg) to rats. The brain concentrations of both drugs increased in a dose dependent manner; those for **2** were about 2—3 times higher than those of sulpiride until 4 h after oral administration (100 mg/kg). The apparent permeability rate constant from

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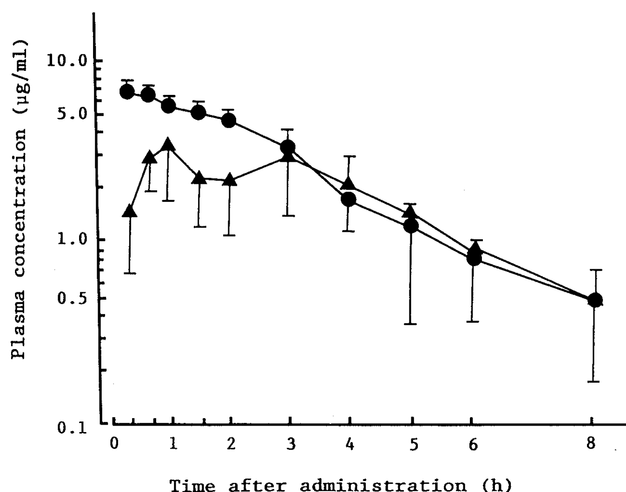

Fig. 3. Plasma Concentration–Time Curves of **2** and Sulpiride after Oral Administration

Symbols are the same as in Fig. 2. Each point represents the mean of 4 or 5 rats and vertical bar indicates S.D. The drugs were administered at a dose of 200 mg/kg.

Table II. Systemic Bioavailability (*BA*) and Partition Coefficients (K_p) of **1** and Sulpiride

Drug	Parameter	
	$BA^{a)}$	K_p
1	60.9±10.9	$6.3^{b)}$
Sulpiride	18.2±6.4	0.2

a) Each value represents the mean±S.D. of 4 or 5 rats. *b*) Data show as compound **2**.

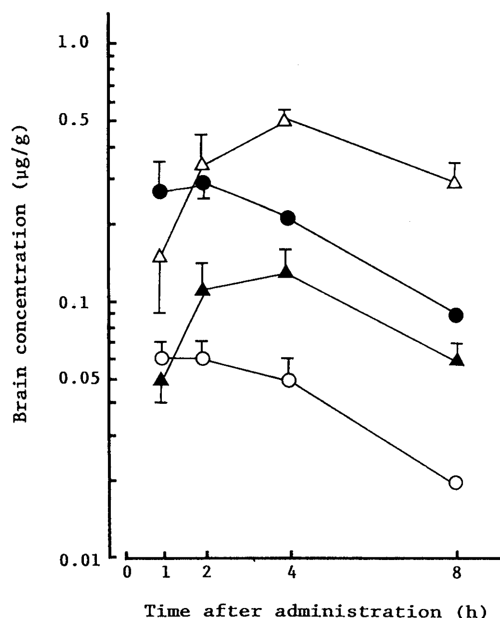

Fig. 4. Brain Concentration–Time Curves of **2** and Sulpiride after Oral Administration

○, **2** (30 mg/kg of **1**); ●, **2** (100 mg/kg of **1**); ▲, sulpiride (100 mg/kg); △, sulpiride (300 mg/kg). Each point represents the mean of 4 rats and vertical bar indicates S.D.

plasma to brain of **2** and sulpiride calculated on the basis of the one compartment open model was 1.226 and 0.560 h^{-1}, respectively. The time required to reach maximum concentration in brain was 1.7 and 3.2 h, respectively. The elimination of **2** from the brain was similar to that of sulpiride. The time courses of the brain/plasma ratios are shown in Fig. 5, and the ratios of the two drugs increased consistently during experiment. The ratios of **2** were about 2 times higher than those of sulpiride.

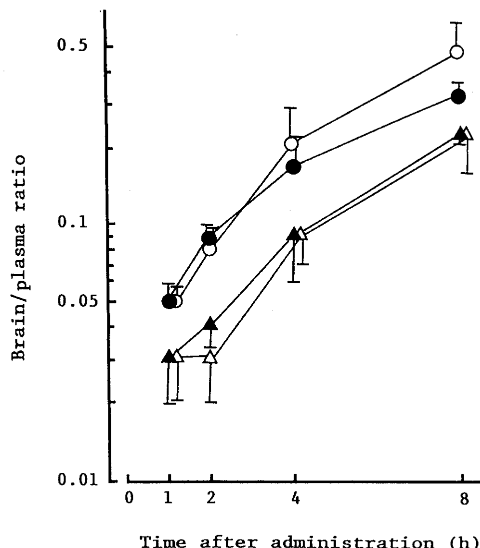

Fig. 5. Brain/Plasma Ratios of **2** and Sulpiride after Oral Administration

Symbols are the same as in Fig. 4. Each point represents the mean of 4 rats and vertical bar indicates S.D.

Discussion

It is well known that membrane permeability of drugs depends on their lipophilicity except for a carrier mediated transport. The K_p values of **2** (the base of **1**) and sulpiride were 6.3 and 0.2, respectively. The value of sulpiride found in this study was similar to that reported previously.[11] This result suggests that **2** is more lipophilic than sulpiride. Reflecting their properties, the pharmacokinetics between **1** and sulpiride were found to be different in rats.

Administered orally, **2** was rapidly absorbed from the gastrointestinal tract, reaching C_{max} at about 0.5 h after dosing. In contrast, the T_{max} (about 1.7 h) of sulpiride was much longer. As presented in Table II, the systemic bioavailabilities of **1** and sulpiride after oral administration were 60.9±10.9 and 18.2±6.4%, respectively. The systemic bioavailability of sulpiride was low, indicating a first-pass metabolism and a poor absorption through the gastrointestinal membrane. Recently, Mizuno and his colleagues[12] demonstrated that the bioavailability of sulpiride after oral administration of sulpiride solution at a dose of 200 mg/kg to rats is 15.6±5.9%, which was in good agreement with our results. Moreover, this low bioavailability was concluded to result not from metabolism in the liver, but from reduced absorption by the gastrointestinal tract. These findings suggest that the rate and extent of bioavailability of **1** are superior to those of sulpiride.

Benakis *et al.*[13] reported that sulpiride concentrations in the brain of rats given ^{14}C-sulpiride were negligible. In this study, the HPLC method was applied to the determination of the brain concentration of **2** and sulpiride, and these concentrations were found proportional to the administered dose. The concentrations of **2** were about 2—3 times higher

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than those of sulpiride. In addition, the rate of permeability of **2** through the blood-brain barrier was faster than that of sulpiride. Recently, the importance of lipophilicity on the delivery of drugs to the brain has been described by several groups.[14] As mentioned, **2** showed more lipophilic property than sulpiride, suggesting that the more lipophilic drug **2** penetrates the blood-brain barrier more extensively than sulpiride.

Administered orally, **1** ($ED_{50}=48$ mg/kg) was 10 times stronger than sulpiride in inhibiting apomorphine-induced hyperactivity in mice.[7] While administered intravenously or intracerebroventricularly, **1** ($ED_{50}=8.5$ mg/kg i.v. and 0.32 mg/kg i.c.v) was respectively 2 times and one third as strong.[7] The differences in pharmacological activity depending on the administration routes for **1** and sulpiride may be due to the differences in the ability of the two drugs to penetrate the gastrointestinal membrane and/or the blood-brain barrier.

In conclusion, the penetration of **1** into the systemic circulation or the brain is greater than that of sulpiride. Therefore, **1** is expected to provide a sufficient clinical effect with a relatively smaller than sulpiride.

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