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Improving the Oral Bioavailability of Sulpiride by Sodium Oleate in Rabbits

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Abstract

To improve the limited oral bioavailability of sulpiride, a dosage form containing sodium oleate as an absorption enhancer was developed and evaluated using gastric-emptying-controlled rabbits in a cross-over manner.

In addition to the known properties of sodium oleate with respect to modifying the permeability of biomembranes, it was found to be capable of improving the physicochemical properties of sulpiride toward a higher lipophilicity (by ion-pair association) and a higher solubility (by micellar solubilization). Nonetheless, the incorporation of sodium oleate with sulpiride as a mixture filled in hard gelatin capsules failed to increase intestinal absorption, whereas the use of enteric capsules, instead of the hard gelatin capsules resulted in a significant increase ($P < 0.05$) in the oral bioavailability.

Sulpiride, a specific antipsychotic drug, is a substituted benzamide. It is usually administered intramuscularly or perorally in the treatment of depression, schizophrenia, and other related psychological disorders. It also finds use in the treatment of migraine, vertigo and certain gastrointestinal disorders (ulcers, irritable colon) (Reynolds 1993).

Many reports have indicated that sulpiride has oral bioavailability problems including differences between animal species, erratic limited absorption, and a high dependence on formulating factors. For example, the bioavailabilities reported in rats and man after oral administration were about 15 and 30%, respectively (Mizuno et al 1986; Bressolle et al 1992), while in dogs the oral bioavailability was about 60% (Segura et al 1976). Kleimola et al (1976) reported that the oral bioavailability in man for sulpiride liquid preparations was significantly less than that for tablets and capsules. Moreover, Gouda et al (1987) suggested that the delay in gastrointestinal transit time due to food or propantheline bromide allows for an improvement in the absorption of sulpiride from the human intestine.

In view of the above observations, and recalling that most psychological diseases need an easy and effective term of treatment, it is desirable to improve the oral availability of sulpiride preparations.

The present study reports an attempt to improve the bioavailability of sulpiride using sodium oleate as an absorption enhancer.

Materials and Methods

Materials

(±)-Sulpiride and sodium oleate were purchased from Sigma Chemical Co., USA. Dogmatil injection ((±)-sulpiride sulphate) was supplied from Fujisawa Pharmaceutical Co., Japan. Hydroxypropyl methylcellulose acetate succinate capsules (HPMC AS-LG enteric capsules, 2 mm × 8 mm) were obtained from Shin-Etsu Chemical Co., Japan. All other chemicals were of the highest grade available and used without further purification.

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Dosage form preparation

Sodium oleate form in hard gelatin capsules. Sulpiride and five times the amount of sodium oleate (w/w) were completely dissolved in a sufficient amount of methanol, and then the solvent was evaporated using a rotatory evaporator at 40°C. The resulting residue was dried under vacuum at room temperature (21°C) for 24 h. The dried product was forced through a 100-mesh sieve and physically mixed with 30% w/w microcrystalline cellulose which was used as a disintegrator. The final mixture was filled in hard gelatin capsules (JP XII, No. 5). Each rabbit received three capsules containing the administered dose of sulpiride.

Sodium oleate form in enteric capsules. The preparation procedures were exactly the same as those above except that a mixture of sulpiride and twice the amount of sodium oleate was prepared and filled in HPMC AS-LG enteric capsules without the addition of microcrystalline cellulose. Each rabbit received four capsules containing the administered dose.

Original powder in hard gelatin capsules. The original powder of sulpiride was administered as a fine powder (100-mesh) filled in hard gelatin capsules (JP XII, No. 5). Each rabbit received one capsule containing the administered dose.

Original powder in enteric capsules. The preparation procedures were exactly the same as those above except that the original powder of sulpiride was filled in HPMC AS-LG enteric capsules. Each rabbit received four capsules containing the administered dose.

Release studies

The dissolution characteristics of the formulations administered were assessed using the JP XII dissolution apparatus with a rotating basket method operating at 100 rev min^{-1}. The dissolution media employed were JP XII 1st (pH 1·2) and 2nd (pH 6·8) disintegration fluids in the presence of 1% w/v polysorbate 20 to facilitate the dispersion of the oleic acid released, since the latter tends to separate from the aqueous bulk of the dissolution medium. Five hundred millilitres of each medium were maintained at 37°C. An amount of the tested formulation corresponding to 15 mg sulpiride was added to each medium, and then samples of 5 mL were removed at predetermined intervals and filtered through membrane filters (pore size, 0·45 μm). Five millilitres of fresh medium were added to the dissolution vessel immediately to maintain the original volume. The removed samples were analysed by HPLC.

Measurement of apparent partition coefficient in chloroform: aqueous phase in the presence and the absence of sodium oleate

A series of aqueous media with various pH values and similar osmolarities (25–40 mOsmol) was prepared using NaOH or HCl for pH adjustment. Ten micrograms of sulpiride with or without 178·3 μg sodium oleate (equivalent to a 20-fold molar ratio) were dissolved in a 5-mL aqueous phase and vigorously shaken with an equal volume of chloroform for 15 min at room temperature. The concentration of sulpiride in both phases was determined by means of HPLC and the pH-partition coefficient profile was plotted and compared with the theoretical pH-un-ionized fraction profile calculated according to the Henderson-Hasselbach equation.

Determination of sulpiride solubility in the presence of sodium oleate

An excess amount of sulpiride was added to tested solutions of sodium oleate in distilled water at different concentrations. The solutions were equilibrated by magnetic stirring at 37°C for 48 h. Samples were withdrawn and filtered through membrane filters (pore size, 0·45 μm), and the filtrates, after suitable dilution, were analysed by HPLC.

Surface tension measurement

The tensiometry of sulpiride saturated solutions in the presence of different concentrations of sodium oleate was completed in duplicate with the drop volume method at 37°C according to Harkins & Brown (1919). The tested solutions were prepared using the same procedures as the solubility determination.

Animal treatment and dosage form administration

Gastric-emptying-controlled rabbits were prepared by the method of Takahashi et al (1983) in order to resemble the gastric emptying of man. In this method, white male rabbits, 3–3·5 kg, were used. All doses given corresponded to 10 mg sulpiride kg^{-1} body weight and were administered in a cross-over manner. Each formulation was inserted into the stomach of the rabbit with a plastic catheter attached to a syringe. The plastic catheter was inserted through a hole in a wooden bar which held the mouth open in such a way that the catheter passed through the oesophagus into the stomach. The dosage forms which had been fixed to the inserted end of the plastic catheter were pushed out of it with 20 mL water into the stomach interior. For the intravenous administration, sulpiride sulphate solution (Dogmatil Injection) was injected through the ear marginal vein. For both intravenous and oral administration, no water was given for the first 4 h and no food was allowed until the study was over. Plasma samples were collected from the ear veins with a heparinized syringe at predetermined intervals and 1 mL plasma was mixed with 2 mL carbonate buffer (0·2 M Na_2CO_3 – 0·2 M $NaHCO_3$, pH 9·8) and extracted with 6 mL chloroform. Five millilitres of the organic layer were evaporated and reconstituted with 0·2 mL internal standard solution (α-naphthylamine, 5 μg mL^{-1}). The resulting solution was used for HPLC injection.

The area under the plasma concentration–time curve from 0 to 24 h after administration (AUC) was calculated according to the linear trapezoidal rule.

Statistical analysis

The AUC values were subjected to statistical analysis and

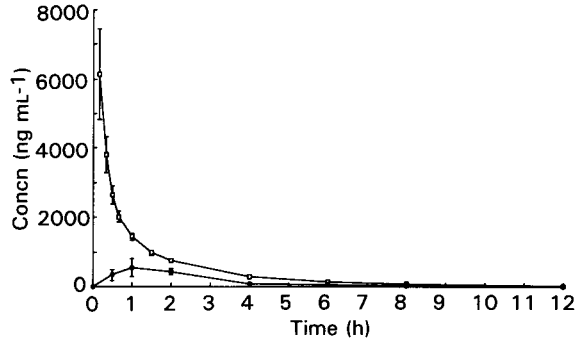

FIG. 1. Sulpiride plasma profiles after intravenous (□) and oral (original powder) (●) administration to rabbits at a dose of 10 mg kg^{-1} in a cross-over study. Each plot represents the mean ± s.e. for three rabbits. The absolute bioavailability of the original powder in hard gelatin capsules was 23%.

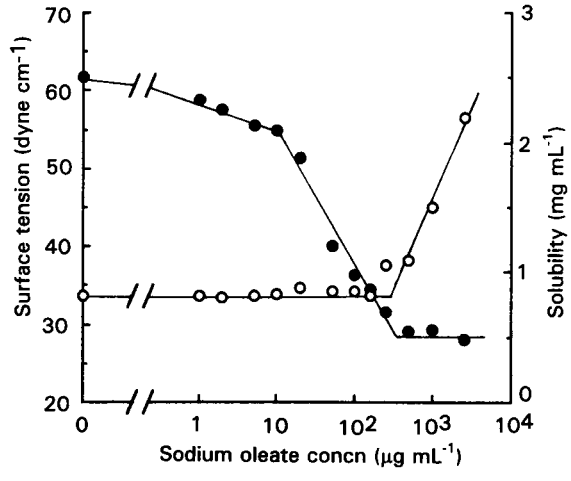

FIG. 2. Simultaneous plot of sulpiride solubility (○) and surface tension (●) as a function of sodium oleate concentration. The measurements were carried out using distilled water at 37°C. The final pH of the saturated solution increased from pH 9·30 (at 0 μg mL^{-1} sodium oleate) to pH 9·61 (at 2500 μg mL^{-1}). Each point represents the mean of two measurements.

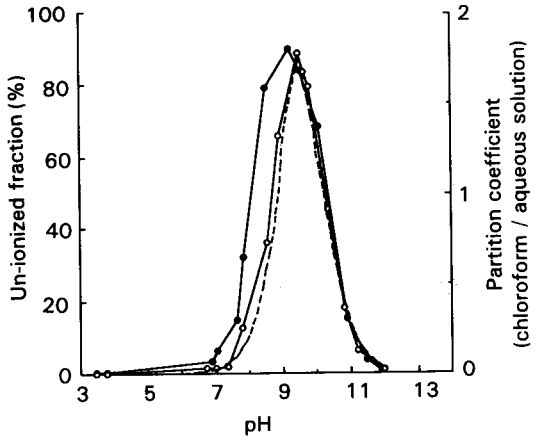

FIG. 3. Sulpiride partition coefficient–pH profile in the presence (●) and absence (○) of sodium oleate at a molar ratio of sulpiride-sodium oleate of 1:20. The dotted line represents the theoretical curve of the un-ionized fraction of sulpiride.

the differences among the treatments were examined by means of Tukey's multiple range test (Tukey 1949).

HPLC conditions for sulpiride determination

A reversed phase ion-pair HPLC method developed by Nicolas et al (1986) was used with minor modifications. A liquid chromatograph (Hitachi 655A-11, Japan) equipped with a fluorescence detector (Jasco 820-FP, Japan) was used. For the stationary phase, a reversed phase column (Hitachi 3053, Japan, 25 cm × 4 mm) was used; the column was warmed at 40°C. The mobile phase consisted of 2·5 mM sodium heptane sulphonate aqueous solution–diethylamine–methanol (60:0·1:40), and pH was adjusted to 3·5 with H_3PO_4. The excitation and emission were set at 300 and 345 nm, respectively. The flow rate was 0·5 mL min^{-1} and the pressure was about 80 kg cm^{-2}.

HPLC conditions for sodium oleate determination

The fluorescent labelling method developed by Nimura & Kinoshita (1980) was used. Briefly, the samples removed from the dissolution vessel were extracted with chloroform, and the resulting extracts were treated with 9-anthryldiazomethane (ADAM). One hour later, the treated extracts were suitably diluted and injected into the HPLC. The stationary phase and other equipment of the HPLC were the same as those which were used for sulpiride determination. The mobile phase was acetonitrile–water (90:10) and the fluorescence was measured at 412 nm, with excitation at 365 nm.

Results and Discussion

Fig. 1 clearly represents a low oral bioavailability in the rabbits studied, which is similar to other bioavailability values reported from man and rats (Mizuno et al 1986; Bressolle et al 1992). Previously, Mizuno et al (1986) reported that the low oral bioavailability of sulpiride in rats was not attributable to liver metabolism. Moreover, in man, Imondi et al (1978) reported that more than 95% sulpiride is renally excreted in an unchanged form following intravenous administration. With respect to rabbits, the present study did not include a special evaluation of sulpiride metabolism. However, comparing the bioavailabilities of a series of different release formulations in rabbits, it was found that the more prolonged release form did not decrease the area under the drug plasma concentration–time curve when compared with the fast release form (data not shown). Hence, also in rabbits, sulpiride will probably not undergo a marked first-pass metabolism. In this case, the poor oral bioavailability seems to be attributable either to the solubility or to the permeability across the biomembrane. In this study, we selected sodium oleate as an enhancer for sulpiride absorption since the effect of oleic acid in terms of disturbing biomembranes had been extensively investigated and was confirmed to be extensive, reversible, concentration–dependent, and relatively harmless (Muranishi 1990). In fact, apart from the above mentioned effects, in this study we found that sodium oleate also improved the physicochemical properties of sulpiride (solubility and partition coefficient) and made it more easily absorbable.

First, we studied the solubility of sulpiride in distilled water in the presence of sodium oleate. It was found that the solubility of sulpiride increased with an increasing sodium oleate concentration when it was above approximately 100 $\mu g\,mL^{-1}$. This increase is probably due to the micellar solubilization, since a simultaneous plot of the surface tension and solubility as a function of log sodium oleate concentration shows slope changes at similar concentrations of sodium oleate (Fig. 2). These changes could be attributed to the critical micelle concentration of sodium oleate under the experimental conditions presented in this study. The logarithmic scale of the sodium oleate concentration was merely used to make the early changes in the slope more noticeable.

Concerning permeability across the biomembrane, we estimated the partitioning of sulpiride between the aqueous media and chloroform and found that sulpiride has a bell-shaped partition–pH profile (Fig. 3). This is assumed to be due to the two ionizable species in the structure of sulpiride,

Table 1. Area under the plasma concentration–time curve (0–24 h) following oral administration of different formulations of sulpiride to rabbits in a cross-over study.

Form administered	AUC (ng h mL^{-1})
Original powder in hard gelatin capsules	1284·7 ± 241·7
Original powder in enteric capsules	961·0 ± 144·6
Mixture with sodium oleate in hard gelatin capsules	741·0 ± 102·3
Mixture with sodium oleate in enteric capsules	2466·4 ± 249·6*

Dose, 10 mg kg^{-1}. Each value represents the mean ± s.e.m. (n = 3). * Significantly different from other forms at $P < 0·05$.

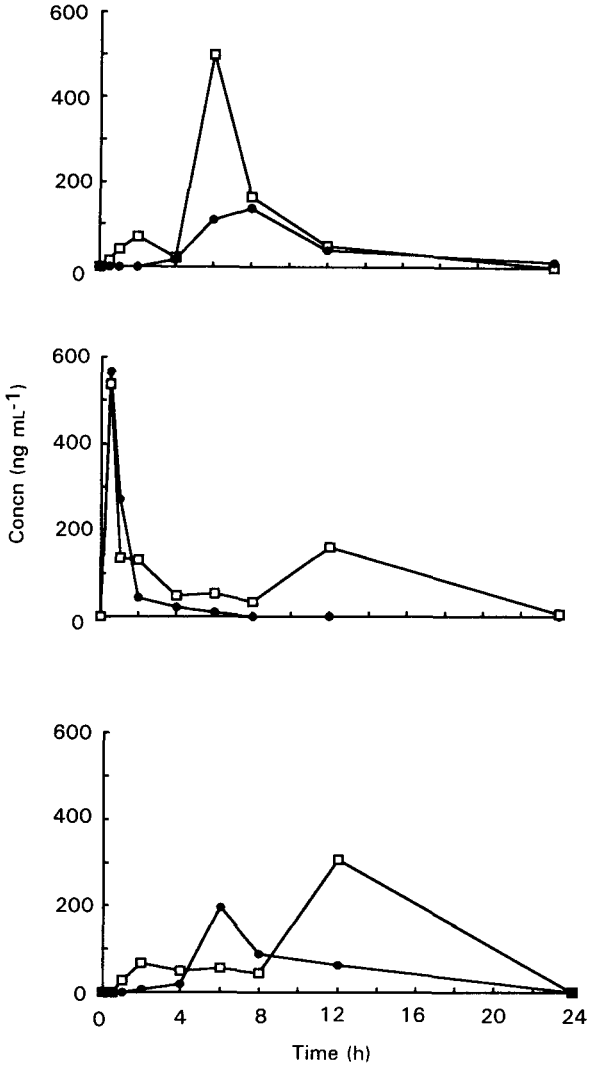

FIG. 4. Individual plasma profiles after oral administration of enteric capsules containing sulpiride alone (●) or sulpiride mixed with twice the amount of sodium oleate (□) to each rabbit at a dose of 10 mg kg^{-1} in a cross-over study.

the nitrogen of the pyrrolidine ring (pK_{a1} 8·99) and the sulphamoyl group (pK_{a2} 10·19) (Davide et al 1988). It is clear from the partition-pH profile that sulpiride is extremely hydrophilic at physiological pH and it is unlikely to be partitioned into lipophilic media.

We studied the effect of sodium oleate on the apparent partition coefficient of sulpiride between the chloroform and aqueous phase. As shown in Fig. 3, there is a shift to the left in the portion of the curve below pH 9·8, reflecting an increase in the partition coefficient. It is worth noting that the left portion of the profile (below pH 9·8) represents the cationic form of sulpiride; therefore, it is possible to assume that the interaction between the cationic form of sulpiride and the oleate anion has an electrostatic nature. Further, the increase in the partition coefficient does not appear to be attributable to the co-solvent mechanism, as the apparent pK_a of oleic acid was reported to be 5·35 (Johns & Bates 1970), and at low pH the oleate will be present in its unionized form; thus, if oleate were acting as a co-solvent, it would increase the partition coefficient even at pH lower

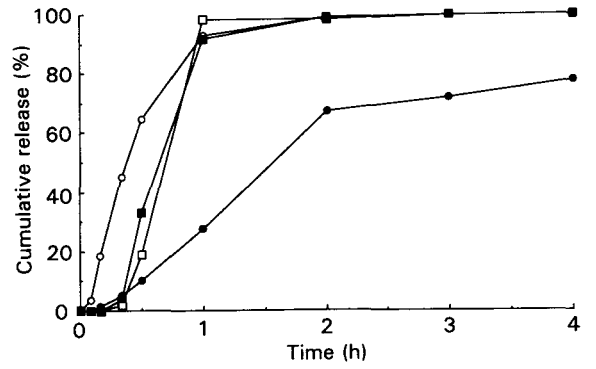

FIG. 5. Release profiles of sulpiride (open symbols) and sodium oleate (closed symbols) from the non-enteric form in JP XII 1st (pH 1·2) (○, ●) and from the enteric form in JP XII 2nd (pH 6·8) (□, ■) disintegration fluids in the presence of 1% polysorbate 20 at 37°C. The non-enteric form is a mixture of sulpiride with five times the amount of sodium oleate and 30% w/w microcrystalline cellulose filled in hard gelatin capsules. The enteric form is a mixture of sulpiride with twice the amount of sodium oleate filled in HPMC AS-LG enteric capsules. Each point represents the mean of two or three measurements.

than 5·35. However, this was not the case, as is evident from the plot. Such behaviour can be explained on the basis of an ion-pairing mechanism between the cationic form of sulpiride and the oleate anion.

Based on these findings, it is expected that the absorption of sulpiride would increase when administered with sodium oleate, since the latter is capable of increasing the solubility and the partition coefficient of sulpiride in addition to its effect on membrane fluidity.

Nonetheless, in the in-vivo experiment, the incorporation of sodium oleate with sulpiride as a mixture filled in hard gelatin capsules (the non-enteric form) failed to increase the intestinal absorption after oral administration to rabbits (Table 1). The use of enteric capsules (the enteric form) instead of the hard gelatin capsules resulted in a significant increase ($P < 0.05$) in the oral bioavailability (from 2·0 to 3·6 times in comparison with the original powder in enteric capsules) as shown in Fig. 4, although the ratio of sodium oleate to sulpiride in the enteric form (due to the size limitation) is lower than that in the non-enteric form. The AUC value for this enteric form was also significanty higher ($P < 0.05$) than those for other formulations (Table 1).

A possible explanation of these results could be provided by comparing the release characteristics of both sulpiride and sodium oleate between the two kinds of formulation. Since sulpiride is a basic drug, it was expected that the release from the non-enteric form would take place mostly in the stomach where the pH is acidic. Therefore, we utilized an acidic medium to determine the release from the non-enteric form and to compare it with that from the enteric form at neutral pH. Fig. 5 reveals that the release rates of both sodium oleate and sulpiride from the enteric form at pH 6·8 are similar and simultaneous, whilst in the case of the non-enteric form, the release rate of sulpiride was higher than that of sodium oleate at pH 1·2. These observations suggest that following the oral administration of the non-enteric form, sufficient dilution and separation of sulpiride from sodium oleate was able to occur before reaching the small intestine, which is considered as the proper site of

absorption. However, as is evident from Fig. 5, the previously mentioned effects (solubility, partitioning membrane, fluidity) are expected to take place more effectively in the case of administering the enteric form in combination with sodium oleate. The present study shows that sodium oleate is effective in enhancing the oral absorption of sulpiride only when it is combined with sulpiride in an enteric form. As a general conclusion, it is suggested that in order to make the effect of an enhancer more apparent, it is preferable to design a dosage form that permits a concomitant and concentrated release of the enhancer along with the drug at the site of absorption.

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