Enhancement of the Oral Bioavailability of Cinnarizine in Oleic Acid in Beagle Dogs

TADAKAZU TOKUMURA*x, YUKI TSUSHIMA*, KIMIO TATSUISHI*, MASANORI KAYANO*, YOSHIHARU MACHIDA‡, AND TSUNEJI NAGAI‡

Received April 7, 1986, from the *Research Center of Technological Development, Eisai Co., Ltd., Minami 2-3-14, Honjo-shi, Saitama 367, Japan, and the ‡Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan. Accepted for publication February 13, 1987.

Abstract □ The present study was an attempt to develop a new dosage form of cinnarizine, which is slightly soluble in water, using lipid as a vehicle. The solubility of cinnarizine in several organic solvents was determined. It was found that cinnarizine dissolved well in oleic and linoleic acids. The bioavailability of cinnarizine from the oral administration of an oleic acid solution in a hard capsule was investigated and compared with that of a cinnarizine tablet, using beagle dogs. When cinnarizine was administered in a capsule, the bioavailability was greatly enhanced [i.e., the maximum concentration (C_{max}) and AUC values were 2.9 and 4.0 times larger than those of a cinnarizine tablet, respectively]. Meanwhile, the t_{max} value (the time to reach C_{max}) was unchanged. The absorption of cinnarizine from an oleic acid solution was considered to depend on the action of bile salts. This was supported by the results of a dissolution test using a bile salts solution as the dissolution test medium.

Cinnarizine [1-cinnamyl-4-(diphenylmethyl)piperazine], an agent for increasing cerebral blood flow, is widely used orally for various problems such as cerebral apoplexy, cerebral arteriosclerosis, and post-traumatic cerebral symptoms. However, cinnarizine is a weak base (pK_{a1} = 1.94[1] and pK_{a2} = 7.47[2]) and its solubility in water is very poor.[3] It is known that the bioavailability of a slightly soluble drug is affected by its dissolution rate in the gastrointestinal tract.[4] This property often brings about problems of efficacy and safety.[5] It has already been reported that the dissolution rate of cinnarizine differs among commercially available pharmaceutical preparations,[6] and that this difference affects the absorption of cinnarizine after oral administration.[6] We previously investigated the enhancement of the dissolution rate and bioavailability of cinnarizine and found that the dissolution rate was increased remarkably by forming an inclusion complex with β-cyclodextrin. The bioavailability was improved when the inclusion complex and a competing agent were administered simultaneously.[7-10]

On the other hand, it was recently reported that the bioavailability of a slightly water-soluble drug (e.g., phenytoin[11] and griseofulvine[12]) was improved by administration with lipid. Therefore, we tried to enhance the bioavailability of cinnarizine by preparing a new dosage form containing lipid as a vehicle.

Experimental Section

Materials—Cinnarizine [1-cinnamyl-4-(diphenylmethyl)piperazine, lot SM 23870] and sodium glycochenodeoxycholate (98% pure, lot 01901) were obtained from Eisai Co., Ltd., and Midori Chemical, respectively. All other chemicals and solvents used were of analytical reagent grade. The oleic acid solution of cinnarizine was prepared by dissolving 50 mg of cinnarizine in 450 mg of oleic acid.

Solubility Study—*In Oily Solvent*—Two grams of cinnarizine was added to 5 g of a solvent in a centrifuge tube which was then sealed and shaken at 25 °C. The solvents used were as follows: sorbitan sesquioleate; polyoxyethylene sorbitan mono-oleate; squalene; cottonseed oil; oleic acid; octyldecyl triglyceride; propylene glycol; and linoleic acid. To achieve equilibrium, the sample solution was occasionally ultrasonicated for 5 min. After equilibration (3 d), an aliquot was filtered with a Toyo No. 2 filter. The concentration of cinnarizine in the filtrate was determined by the ultraviolet (UV) absorption method using a Hitachi EPS-032 spectrophotometer.

Effect of Sodium Glycochenodeoxycholate and Oleic Acid—Cinnarizine (250 mg), with or without 7.5 mg of oleic acid, was added to 15 mL of the second fluid of the Japanese Pharmacopoeia X (JP X) disintegration test procedure containing 20 mM sodium glycochenodeoxycholate. After equilibration (3 d) at 37 °C, an aliquot was filtered with a disposable filter (Acrodisc, 0.2 μm, German Co., Ltd.). The concentration of cinnarizine in the filtrate was determined by an HPLC method similar to that used in the absorption study.

Absorption Study—Four male beagle dogs were used after they had fasted for 18 h. A gelatin capsule, filled with 500 mg of sample solution containing 50 mg of cinnarizine dissolved in 450 mg of oleic acid, was administered to the dogs with ~30 mL of water. A 2.5-mL blood sample was taken from each dog's cephalic vein at 0.5, 1, 2, 4, 6, and 8 h after oral administration. The blood samples were centrifuged for 10 min at 3000 rpm using an Hitachi HIMAC centrifuge (SCR 20B). In each case, the plasma layer was removed and frozen at −20 °C until the time of analysis. The determination of cinnarizine in the plasma was carried out according to the following method. One milliliter of the plasma sample, 0.5 mL of 1 M HCl, and 5 mL of ether were added to a glass-stoppered centrifuge tube which was shaken well and centrifuged. After the organic layer was discarded, the aqueous layer was extracted with 5 mL of dichloromethane, and the dichloromethane phase was evaporated to dryness. The residue was dissolved in 100 μL of mobile phase [acetonitrile:0.01 M $NH_4H_2PO_4$ (70:30)], which contained 100 μg of meclizine hydrochloride as an internal standard, and 50 μL of the solution was injected into a Shimadzu LC-5A HPLC. The eluant was monitored spectrofluorometrically by using a fluorescence monitor (Shimadzu RF-530). The excitation and emission wavelengths were 260 and 315 nm, respectively. A column of Nucleosil C_{18} (5-μm particle size, 4 mm × 25 cm) was used for the analysis.

Procedure for Dissolution Study—The dissolution profiles of cinnarizine from a tablet containing 25 mg of cinnarizine and a capsule containing 250 mg of oleic acid solution with 25 mg of cinnarizine were determined by the "paddle method" that is described in the JP X. The dissolution medium was the second fluid of the JP X disintegration test procedure with 20 mM sodium glycochenodeoxycholate,[13] and its pH was 6.8 before and after the dissolution test. The dissolution test was performed using 900 mL of the medium at 37 °C at a stirring speed of 100 rpm. The amount of cinnarizine dissolved was measured by an HPLC method similar to that used in the absorption study.

Results and Discussion

Solubility in Oily Solvents—Table I shows the solubility of cinnarizine in several solvents. It was found that cinnarizine dissolved well in oleic and linoleic acids. Oleic and linoleic acids are unsaturated fatty acids and have been known to easily form a micelle with bile salts in the gastroin-

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Table I—Solubility of Cinnarizine

Solvent	Solubility, % w/w
Sorbitan sesquioleate	2.1
Polyoxyethylene sorbitan monooleate	0.9
Squalene	1.7
Cottonseed oil	1.2
Oleic acid	23.7
Octyldecyl triglyceride	1.8
Propylene glycol	1.9
Linoleic acid	21.3

testinal tract.[14,15] Therefore, oleic acid was chosen as the solvent for cinnarizine in the preparations administered to dogs. When cinnarizine is administered with these two solvents, cinnarizine may form a mixed micelle with these solvents and bile salts. Hence, an increase of absorption may be expected.

Absorption Study—Figure 1 shows the mean plasma level of cinnarizine after the oral administration of an oleic acid solution of cinnarizine. The plasma level was significantly increased compared with that of the administration of a cinnarizine tablet. The bioavailability parameters, area under the concentration–time curve (AUC) and the maximum concentration (C_{max}), were also increased, as shown in Table II. The AUC and C_{max} of the oleic acid solution were 4.0 and 2.9 times larger than those of the cinnarizine tablet, respectively. The value of the time to reach C_{max} (t_{max}) was not changed. It has been reported that oleic acid delays gastric emptying in rats;[11] however, this phenomenon was not observed in this experiment. This difference seems to be due to the different species of animal used.

These results indicate that the administration of cinnarizine in an oleic acid solution greatly increases the bioavailability of cinnarizine. The absorption process of cinnarizine from the oleic acid solution will differ from the absorption process from the tablet. Because cinnarizine is a slightly water-soluble drug and its partition coefficient is larger even in a low pH range,[3] it is difficult to consider that the cinnarizine was absorbed after the partition from an oleic acid to the aqueous phase in the gastrointestinal tract.

The absorption of a water-insoluble and oily drug, such as tocopherol and phytonadione, is known to depend on the action of bile salts. From this point of view, an evaluation method for a water-insoluble and oily drug preparation has been developed as a dissolution test using a bile salts solution.[13] Oleic acid is known to form a mixed micelle with bile salts in the gastrointestinal tract. When cinnarizine was administered as an oleic acid solution, it was speculated that a mixed micelle of cinnarizine with oleic acid and bile salts was formed in the gastrointestinal tract. Therefore, in order to confirm the effect of the bile salts on the absorption of cinnarizine from the oleic acid solution, a dissolution test using a bile salts solution was performed.

Dissolution Rate—Figure 2 shows the results of the dissolution test for a cinnarizine tablet and a capsule containing the oleic acid solution of cinnarizine. The dissolution rate from the capsule was clearly more rapid compared with that from the tablet. This result indicates that cinnarizine in oleic acid solution is easily affected by bile salts. In addition, this was supported by the result of the solubility study. The solubility of cinnarizine in 20 mM sodium glycochenodeoxycholate (30.4 μg/mL) was increased by the addition of oleic acid (40.7 μg/mL). This phenomenon was considered to be brought about by the formation of a mixed micelle of cinnarizine, oleic acid, and sodium glycochenodeoxycholate. From these results, it was confirmed that oleic acid and bile salts affect the absorption of cinnarizine by forming a mixed micelle.

In the results of the dissolution test, a small dissolution rate from the tablet was observed. This suggests that the bile salts affect the absorption of cinnarizine from the tablet form. However, the absorption of cinnarizine from the tablet depended on the acidity in the stomach, and the effect of bile salts or bile was not observed in the absorption study using beagle dogs under fasted conditions.[3] This may have been because the amount of bile salts in the small intestine was little, that is, not enough to affect the absorption of cinnarizine.

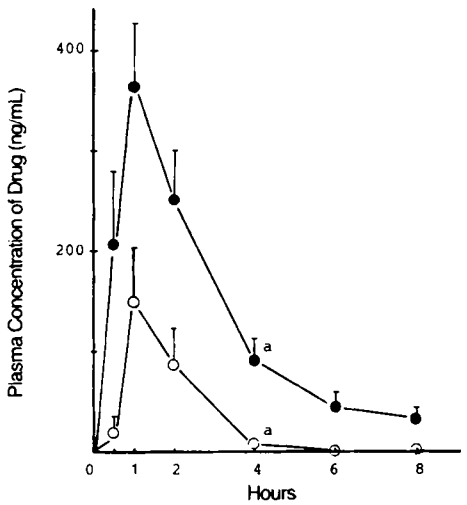

Figure 1—*Plasma concentration of cinnarizine after the oral administration of* (○) *a cinnarizine tablet (ref 10), and* (●) *the oleic acid solution of cinnarizine. Each point represents the mean ± SEM of 3–4 dogs; points marked* a *have a* $p < 0.05$.

Table II—Bioavailability Parameters of Cinnarizine after Oral Administration of Tablet and Oleic Acid Solution of Cinnarizine

	$AUC_{0-8\ h}$, $ng \cdot h \cdot mL^{-1}$	C_{max}, ng/mL	t_{max}, h
Tablet[a]	267.2 ± 102.9[b,c]	145.3 ± 55.3[c]	1.0
Oleic acid solution	1064.0 ± 91.7[c]	417.7 ± 30.2[c]	1.5 ± 0.3

[a] Data from ref 10. [b] Each value represents the mean ± SEM of 3–4 dogs. [c] $p < 0.01$.

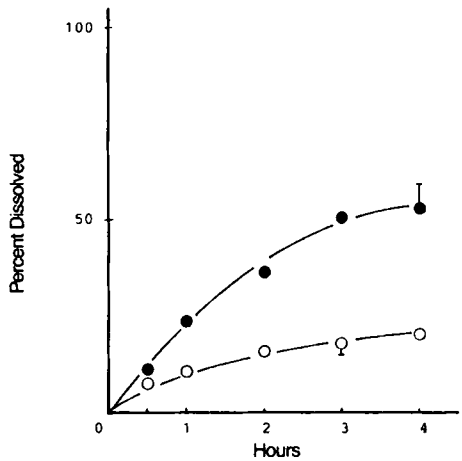

Figure 2—*Dissolution of cinnarizine from both tablet and capsule (containing the oleic acid solution of cinnarizine) dosage forms into a medium containing glycochenodeoxycholate. Key:* (○) *tablet;* (●) *capsule. Each point represents the mean ± SEM of three determinations.*

The increasing bioavailability of cinnarizine from the oleic acid solution seemed to be caused by the action of bile salts on oleic acid and cinnarizine. On the other hand, various physiological mechanisms have been proposed to explain the effect of oil on the absorption of drugs; for example, altered gastrointestinal motility, increased bile flow and drug solubilization,[16] increased mucosal permeability,[17] enhanced mesenteric lymph flow,[18] and increased lymphatic absorption.[19]

In the case of the dosage form of cinnarizine used in this study (using oleic acid as a vehicle) and considering the above mechanisms, the increasing effect of oleic acid on bile flow or mucosal permeability might be expected to increase the bioavailability of cinnarizine. In addition, several absorption processes of drugs from the micelle phase were reported by Ogata et al.[20] It is almost certain that the increasing bioavailability of cinnarizine observed in this study was achieved by changing the absorption process to include the formation of mixed micelles, which depends on the action of oleic acid and bile salts. However, the effect of oleic acid on the increasing bioavailability of cinnarizine or the cinnarizine absorption process from the mixed micelle is not clear. Therefore, further investigation is necessary to resolve these problems.

In conclusion, cinnarizine is a slightly water-soluble drug whose absorption depends on the dissolution rate of the preparation in the gastrointestinal tract. However, the dosage form used in this study, using oleic acid as a vehicle, was thought to be absorbed through a mixed micelle formation of cinnarizine with oleic acid and bile salts. This dosage form seems to be effective for the enhancement of the bioavailability of slightly water-soluble drugs. These drugs generally dissolve well in organic solvents, such as oleic acid, and drugs dissolved in organic solvents are considered to easily form a micelle with bile salts. In addition, a dissolution test, using a bile salts solution, was found to be a good method for the evaluation of this dosage form.

References and Notes

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