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Bioavailability of itazigrel in dogs after oral administration of soft elastic capsules

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Summary

The effect of Tween 80, a solubilizing agent, on gastrointestinal absorption was investigated in dogs after oral administration of soft elastic capsules (SEC) of itazigrel with respect to bioavailability. The bioavailability was determined from the area under the curve (AUC) of plasma itazigrel concentration after drug administration. In spite of its known low water solubility (less than 100 ng/ml), the mean absolute bioavailability of itazigrel was about 90% in six dogs under fasting conditions. Furthermore, the absolute bioavailability was also about 85% when measured 30 min after standard food was given to the dogs. The high bioavailability observed in this oral study is believed to be due to Tween 80 contained in SEC which apparently caused complete dissolution in the gastrointestinal tract.

Introduction

Itazigrel (2-trifluoromethyl-4,5-bis[*p*-methoxyphenyl]thiazole) is an inhibitor of cyclooxygenase activity and collagen-induced platelet aggregation (Nishizawa et al., 1982). As such, it is thought to have clinical applications in arterial and microvascular diseases and for preventing venous thrombosis and pulmonary embolism. As itazigrel has extremely low water solubility, it is likely to present poor bioavailability after oral administration (Fincher, 1968). In order to overcome this problem, a wide range of techniques have been investigated for the preparation of solid dispersions (Sugimoto et al., 1980; Jachowicz, 1987; Nishihata et al., 1987). However, the task of producing reproducible batches of solid dispersions was difficult. The use of solubilizing agents such as polysorbate 80 (nonionic surfactant, hereinafter referred to as Tween 80) or other oils has also been widely investigated with the purpose of increasing the oral bioavailability of poorly water soluble drugs (Amidon et al., 1982). Because of the physicochemical nature of itazigrel with its extremely low water solubility and obvious concern regarding drug bioavailability, a soft elastic capsule (SEC) formulation containing Tween 80 as a vehicle appears to be a candidate for use as a dosage form.

In the present study, we investigated plasma itazigrel concentrations in dogs after oral admin-

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istration of SEC to determine the bioavailability, in comparison with the concentrations after intravenous administration.

Materials and Methods

Materials and formulations

Itazigrel was supplied by The Upjohn Co. (MI, U.S.A.). Tween 80 was obtained from Nikko Chemicals (Tokyo, Japan). Other reagents used were of analytical grade. SECs were manufactured by R.P. Sherer (Tokyo, Japan). Before encapsulation, 25 mg of itazigrel was dissolved in 205 mg of Tween 80. One capsule contained 25 mg of itazigrel.

For intravenous administration, 1 g of itazigrel was dissolved in 100 ml of polyethylene glycol 200, and 1 ml of this solution was dosed intravenously.

Animals

Six male beagle dogs, each weighing between 9.5 and 10.5 kg, were used after fasting for 16 h prior to experiments. At 4 h after dosing, food was given. In the experiment to investigate the effect of food, the dogs were fed with 250 g of LABO D Stock (Nihon Nousan, Tokyo, Japan) 30 min before dosing.

Solubility study

The effect of Tween 80 on the solubility of itazigrel was studied as follows: 1.0 g of itazigrel was added to 5 ml of 0.1 M sodium phosphate buffer at pH 6.8, containing various concentrations of Tween 80 in the test tubes, which were then incubated at 37°C for 48 h. The sample solution was collected through a Millipore filter (0.22 μm pore size) and assayed for itazigrel concentration by high-performance liquid chromatography (HPLC). Further, to investigate the behavior of itazigrel in Tween 80 after dilution in saline, the following experiment was performed: Tween 80 saturated with itazigrel at 37°C was diluted with saline (37°C) in order to provide various concentrations of Tween 80. At 1 and 3 h thereafter, the sample solution was collected through a Millipore filter (0.22 μm) for assay of itazigrel.

In vitro capsule disintegration study

The disintegration study of SECs was performed according to the JP-XII disintegration test by using a disintegration tester with distoper (Toyama Sangyo, Tokyo, Japan) as follows: JP-XII disintegration medium (first fluid) at pH 1.2 was maintained at a constant temperature of 37°C. The stroke rate of the basket was 30 strokes/min. The endpoint was detected by an interception tube crossing the phototube of the apparatus 'distoper'.

In vitro dissolution study

The study was conducted according to the JP-XII paddle method with minor modification. Because of the poor aqueous solubility of itazigrel (less than 100 ng/ml), complete dissolution was not observed in 900 ml of JPXII disintegration medium. Since it is indicated in the USP that a dissolution medium for poorly aqueous soluble drugs must be selected to satisfy the condition under which the solubility of drug in the medium is at least 3-times greater than the concentration of drug completely dissolved in the dissolution medium (900 ml), a solubilizing agent was employed in the present study. One SEC was used in each dissolution test, and three tests were performed for each formulation (Table 1). Upon adding an SEC into 900 ml of the JP-XII disintegration first medium containing 0.5% w/v Tween 80 at 37°C, 0.8 ml of the medium was collected through a Millipore filter (pore size 0.22 μm) at 0, 3, 6, 9, 12, 15, 20, 25, 30, 45, and 60 min.

TABLE 1

In vitro disintegration of SEC and dissolution of itazigrel

Lot no. of SEC	Disintegration endtime (min)	Dissolution	
		Time for 50% dissolution (min)	MDT (min)
RJ533202P	8.6 ± 1.0	18.2 ± 2.5	22.5 ± 0.7
RJ533203P	7.8 ± 0.9	13.8 ± 2.0	18.7 ± 2.9
CJ533203P	7.4 ± 0.8	16.1 ± 1.9	20.5 ± 4.0

Each value represents the value of mean ± S.D. ($n = 6$).

During the experiment, the medium was stirred with a paddle at 100 rpm. After 0.2 ml of the sample solution had been diluted with methanol containing internal standard (U-64899 [4,5-bis(4-ethoxyphenyl)-2-(trifluoromethyl)thiazole], at 200 ng/ml), the mixture was assayed by HPLC.

In vivo absorption study in dogs

Following oral administration of an SEC, 5 ml of blood was collected from the left femoral vein with a heparinized syringe at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h. The blood was centrifuged at 3000 rpm for 10 min to obtain plasma, which was then kept at −20°C until use.

For the experiment by intravenous (i.v.) administration, 5 ml of blood was collected from the right femoral vein at 0, 0.033, 0.083, 0.133, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h. In the i.v. administration study, slight hemolysis was observed in the sample only during the very early stage, however, it was not significant in extent. Just before HPLC assay, 1 ml of methanol was added to 0.5 ml of the plasma, and then 0.5 ml of acetonitrile containing 100 ng of internal standard (U-64899) was added by mixing vigorously for 30 s. After centrifugation at 3000 rpm and 4°C for 30 min, the supernatant was collected for HPLC assay.

Assay of itazigrel

Itazigrel was assayed by HPLC according to the following condition: A liquid chromatograph (Model LC-6A, Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (RF-535, Shimadzu) was used. The separation column (inside diameter 4.6 mm × 15 cm long) contained a reverse phase (LiChro-CART 125–4, RP 8-e end-capped, Merck,. U.S.A.). The mobile phase was a mixture of 85 ml methanol and 15 ml distilled water. The flow rate was 0.8 ml/min. Itazigrel was monitored by fluorescence detection at 320 nm for excitation and 430 nm for emission. The retention time of itazigrel and internal standard (U-64899) was 6.9 and 11.3 min, respectively. The assay limit for itazigrel was 10 ng/ml in plasma samples. Regarding the reproducibility of the assay, the mean results ±S.D. were 9.93 ± 0.36, 41.1 ± 0.90, 104 ± 4.2, 389 ± 2.7 ng/ml for 10.0, 40.0, 100 and 400 ng/ml of itazigrel, respectively.

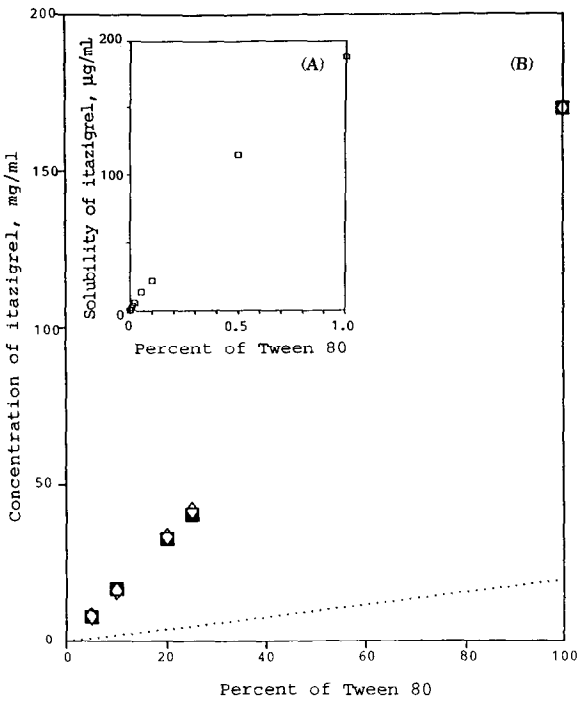

Fig. 1. (A) The effect of Tween 80 on the solubility of itazigrel in pH 6.8 sodium phosphate buffer and (B) the concentration of itazigrel at 1 h (■) and 3 h (◇) after dilution of Tween 80 vehicle saturated with itazigrel using saline. Dashed line in (B) represents the concentration of itazigrel estimated from the data in (A).

Statistical analysis

Statistical analysis was carried out by ANOVA analysis.

Results

Solubility of itazigrel in aqueous medium containing various concentrations of Tween 80

The solubility of itazigrel powder in phosphate buffer (pH 6.8) which contained Tween 80 (0.002–1% w/v) increased linearly as the concentration of Tween 80 increased (Fig. 1A). During this experiment, the sample collected through the Millipore filter was a clear solution.

When Tween 80 saturated with itazigrel was diluted with saline to a concentration of Tween

80 of 5–25%, the concentration of itazigrel in the aqueous medium decreased linearly along with the increase in dilution ratio with saline after filtration through the Millipore filter at 1 and 3 h after mixing (Fig. 1B), i.e., itazigrel appeared to have precipitated very little during the experimental period of 3 h. The sample collected after filtration was white and turbid, i.e., an emulsion seemed to have been formed. This method was employed to estimate the precipitation of itazigrel in the fluid in the gastrointestinal tract after oral administration of itazigrel SECs.

Disintegration of SECs and dissolution of itazigrel

No significant difference was observed in the endtime of in vitro disintegration among the three SEC lots manufactured (Table 1). Fig. 2 shows the in vitro dissolution profiles of SECs. No significant differences were observed in fractional dissolution at 15, 30, 45, and 60 min among the three lots manufactured. To characterize the in vitro dissolution, a model-independent method based on moment analysis (Tanigawara et al., 1982a) was employed, thereby defining the dissolution of drug based on the mean in vitro dissolution time (MDT) according to Eqn 1:

$$\mathrm{MDT} = \int_0^\infty t(\mathrm{d}m/\mathrm{d}t)\mathrm{d}t \Big/ \int_0^\infty (\mathrm{d}m/\mathrm{d}t)\mathrm{d}t \qquad (1)$$

where m is the mass of drug dissolved in solution at time t. The MDT was calculated from Eqn 1

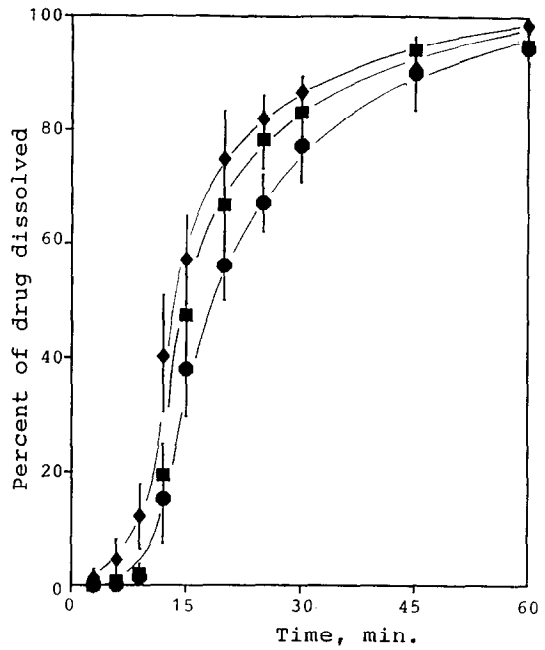

Fig. 2. In vitro dissolution profiles of itazigrel from SECs: (●) SEC of lot no. RJ533202P; (◇) SEC of lot no. RJ533203P; (■) SEC of lot no. CJ533203P. Each value represents the mean ± S.D. ($n = 3$).

by using a personal computer (Table 1). There were no significant differences in MDT and time required for 50% dissolution among the three lots manufactured. As no differences were observed in the in vitro disintegration and in vitro dissolution among the three lots, SEC lot

TABLE 2

Pharmacokinetic parameters of itazigrel after administration in dogs

	i.v. under fasting conditions	p.o. under fasting conditions	p.o. under postprandial conditions
Dose (mg/body)	10	25	25
AUC (ng ml^{-1} h)	529.8 ± 110.1	1231.9 ± 389.4	1187.6 ± 311.1
BA (%)	–	88.1 ± 18.2[a]	85.7 ± 9.5[a]
C_{max} (ng/ml)	–	505.5 ± 160.1	297.9 ± 173.4[b]
T_{max} (ng/ml)	–	0.8 ± 0.2	1.7 ± 0.7[b]
MRT (h)	0.80 ± 0.43	9.77 ± 3.84	9.04 ± 2.27
MAT (h)	–	8.97 ± 3.74	8.24 ± 2.46

[a] Absolute bioavailability was calculated according to Eqn 2 in the text.

[b] $p < 0.05$ vs the value obtained under fasting conditions.

Each value represents the mean ± S.D. ($n = 6$).

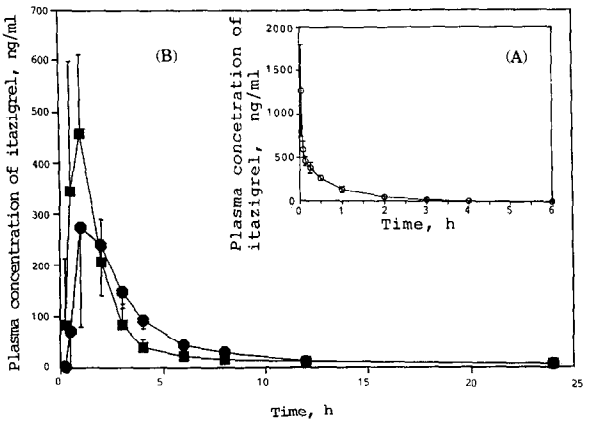

Fig. 3. (A) Plasma itazigrel concentration in dogs under the fasting conditions as a function of time after intravenous administration of itazigrel at a dose of 10 mg/body. (B) Plasma itazigrel concentration in dogs under either fasting condition (■) or post-meal condition (●), as a function of time after oral administration of SEC containing itazigrel of 25 mg/SEC. Each value represents the mean ± S.D. ($n = 6$).

CJ533203P was used in the in vivo absorption study.

In vivo absorption study in dogs

To estimate the absolute bioavailability of itazigrel after oral administration of SECs in the dogs under fasting conditions, the area under the curve (AUC) of the plasma concentration of itazigrel was calculated using the trapezoidal integration method. The AUC for infinite time was obtained by single-exponential extrapolation of the terminal phase of the plasma itazigrel concentration curve. The AUC after oral administration was compared to that after i.v. administration (Table 2).

After oral administration of SEC, a rapid increase in plasma itazigrel concentration was observed, followed by an initial rapid disappearance; T_{max} was about 1 h (Fig. 3 and Table 2). The absolute bioavailability (BA) of itazigrel after oral administration, which was calculated according to Eqn 2, was about 90% (Table 2):

$$BA = (AUC)_{or}(dose)_{i.v}/(AUC)_{i.v.}(dose)_{or} \quad (2)$$

To characterize the biopharmaceutics of itazigrel, a model-independent method, based on moment analysis, was employed as reported by Yamaoka et al. (1978). In this method, the behavior of a drug in plasma was defined by the mean in vivo residence time (MRT) determined using Eqn 3:

$$MRT = \int_0^\infty tC_p dt / \int_0^\infty C_p dt = AUMC/AUC \quad (3)$$

where C_p is the drug concentration in plasma at time t. The MRT was obtained from the AUC and the area under the first moment curve (AUMC) for infinite time. The AUMC to the last measured plasma concentration was calculated based on the linear trapezoidal rule with addition of the correction term after the last measured point to infinity. It has also been reported by Tanigawara et al. (1982b) that the mean absorption time (MAT) can be estimated with Eqn 4:

$$MAT = MRT_{or} - MRT_{i.v.} \quad (4)$$

where MRT_{or} and $MRT_{i.v.}$ are the MRT after oral and intravenous administration, respectively. A longer MRT after oral administration under fasting conditions was observed in comparison to that after intravenous administration (Table 2).

The AUC of itazigrel after oral administration in the dogs to which food was given 30 min before SEC administration did not show a significant difference from that in dogs under fasting conditions, i.e., the absolute bioavailability was about 85% (Table 2). When the dogs were fed before dosing, the C_{max} decreased and T_{max} was prolonged compared to those in dogs which were fasting (Table 2). A longer MRT was also observed after oral administration under postprandial conditions as seen in the case of fasting conditions, however, no significant difference between the two conditions of MRT and MAT was observed (Table 2).

Discussion

In spite of its low water solubility (less than 100 ng/ml), itazigrel was completely dissolved in the presence of Tween 80 in this in vitro dissolution study (Fig. 1). Because Tween 80 in the medium acts as a solubilizing agent, it apparently

caused the complete dissolution of itazigrel after it had been released from the SECs. The use of Tween 80 as a formulation vehicle improved the dissolution step of itazigrel, by overcoming the poor aqueous solubility of the drug, which was otherwise considered to be the rate-limiting factor in the overall absorption process.

It is known that Tween 80 forms micelle at low concentrations in water and increases the apparent solubility of poorly water soluble drugs (Park and Rippie, 1977). It has also been reported that the increase in solubility relates to the ratio of Tween 80 content in water (Park and Rippie, 1977), i.e., the drug distributes to both water medium and micelles of Tween 80. Thus, the increase in solubility of drug in the medium containing Tween 80 occurs linearly along with the increase in Tween 80 in the medium, as found in the present study. However, the concentration of itazigrel (about 20 mg/ml) in Tween 80 itself estimated from the intercept of the linear portion of the dashed line in Fig. 1B at 100% of Tween 80 is very low in comparison to the solubility of itazigrel determined (180 mg/ml). This discrepancy may indicate that Tween 80 as micelles in water behaves differently in dissolving (incorporating) drug from pure Tween 80. When Tween 80 saturated with itazigrel was diluted with saline, itazigrel apparently precipitated only slightly, probably by formation of an emulsion as described under Results. The cause of the considerable intestinal absorption of itazigrel after oral administration of SEC may be due to the formation of micelles and/or emulsion in the fluid in the gastrointestinal tract. It is also considered that the micelle and/or emulsion formation of Tween 80 acts as a driving force to cause significant dissolution of itazigrel. It has been reported that micelles could act as a carrier of drug across an aqueous diffusion layer to the membrane surface (Amidon et al., 1982), i.e., an increase in the concentration of itazigrel on the mucosal membrane surface even in forming micelles may cause considerable absorption from the intestinal surface. On the other hand, it has often been found that the presence of a surfactant such as Tween 80 causes a delay in intestinal absorption (Levy and Reuning, 1964) or decreases the disappearance from the intestinal lumen in the in situ rat intestinal loop method (Tahara et al., 1993). These surfactant effects were explained as an entrapping effect in the micelle, however, this would only be expected when micelles of a surfactant are stable in the intestinal fluid.

Since we have recently observed that the in vitro release of itazigrel entrapped in Tween 80 vehicle was accelerated significantly by esterase which degraded Tween 80 (Nishihata et al., 1993), the rapid absorption and high bioavailability of itazigrel observed in dogs after oral dosing of the SECs may also be related to the action of esterase in the small intestinal tract, i.e., the high bioavailability of itazigrel (Table 2 and Fig. 3) after oral administration of SECs in the fasting dogs seems to have been induced by the effect of Tween 80 apparently by causing the apparent complete dissolution of itazigrel in the gastrointestinal tract (Fig. 1) and probably by effective release from the micelles by esterase action (Nishihata et al., 1993). Thus, the SEC formulation investigated in this study appears to be an effective oral formulation for poorly water-soluble drugs which often present low bioavailability.

Furthermore, there are several reports describing that the intestinal absorption of poorly water-soluble drugs after oral administration is increased markedly after meals (Aoyagi et al., 1982). Food results in an increase of bile secretion into the intestinal tract and the bile acts to solubilize the poorly water-soluble drug, i.e., bile increases the dissolution rate of poorly water-soluble drugs. In the present study, no significant difference was observed in the bioavailability of itazigrel after oral administration irrespective of whether or not the dogs were fed prior to treatment. The amount of Tween 80 in the vehicle seems to be sufficient to lead to the apparently complete dissolution of itazigrel even after dilution by gastrointestinal fluid.

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