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Enhanced rectal absorption of itazigrel formulated with polysorbate 80 micelle vehicle in rat: role of co-administered esterase

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Abstract—We investigated the effect of esterase on rectal absorption in the rat of itazigrel using polysorbate 80 (PS-80) micelle as a vehicle to overcome the poor aqueous solubility of itazigrel. The itazigrel formulation prepared with PS-80 increased the absorption compared with a 0·25% carmellose sodium suspension, probably by supplying the itazigrel solute to keep a high concentration at the epithelial surface. When esterase was co-administered with the formulations containing PS-80, the absorption of itazigrel from rat rectum was accelerated further, by rapid release of itazigrel from the micelle vehicle after enzymatic degradation of the PS-80 micelle.

Generally, lipophilic drugs are easily absorbed from the intestinal tract after they have dissolved in intestinal fluid. Dissolution is rate-limiting in intestinal absorption for poorly water-soluble drugs even with high lipophilicity (Fincher 1968; Nishihata et al 1988). It has been reported that solubilizing agents, including polysorbate 80 (PS-80) enhanced intestinal absorption of poorly water-soluble drugs (Amidon et al 1982). Apparently the micelle functioned to overcome the diffusion layer resistance in-vivo, thereby delivering the maximal solute concentration to the membrane surface (Westergaard & Dietschy 1976; Amidon et al 1982). This concept is based on the partition equivalent between the polysorbate micelle vehicle and aqueous medium such as intestinal fluid. However, this technology sometimes decreases the apparent intestinal absorption of certain compounds (Levy & Reuning 1964). We have reported that oleaginous vehicles, including glyceride ester, release drugs incorporated in the vehicles by enzymatic (lipase) degradation (Nishihata et al 1986; Yoshitomi et al 1987). We have also reported that PS-80 vehicles incorporating sudan II (poorly water-soluble dye) released sudan II easily in rat small intestine, in spite of no release in rat stomach, after oral dosing (Nishihata et al 1993); i.e. it was considered that the role of the PS-80 vehicle in increasing intestinal absorption of poorly water-soluble drugs was to transport the drug in solution to the small intestinal tract without precipitation in the stomach and to release drug by enzymatic (small intestinal esterase) degradation of vehicle for intestinal absorption.

To understand the role of PS-80 as the vehicle, it is necessary to clarify the enhancing effect of PS-80 on the intestinal absorption of poorly water-soluble drugs in the absence of enzymatic degradation of PS-80, and then to investigate the role of esterase on the PS-80 vehicle. In the present study, we investigated the effect of PS-80 on the rat rectal absorption of itazigrel, which is known to have poor water solubility (less than 100 ng mL^{-1} (Nishihata et al 1993)), and the effect of esterase co-administered with dosing vehicles containing PS-80.

Materials and methods

Materials. Itazigrel and U-64899 were supplied by The Upjohn Company (Michigan, USA). Polysorbate 80 (PS-80) was obtained from Nikko Chemicals (Tokyo, Japan). Porcine liver esterase (230 units (mg protein)$^{-1}$, EC.3,1,1,1) was obtained from Sigma Chemicals (MO, USA). Other reagents used were of analytical grade.

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Formulations. Table 1 lists the itazigrel formulations used. Formulations 1 to 4 were prepared by diluting PS-80 containing itazigrel (125 or 50 mg g^{-1}) with saline; the saturated concentration of itazigrel in PS-80 was 180 mg g^{-1}. The solution formulation was prepared by dissolving itazigrel in 0·25% w/v oleic acid solution. The suspension formulation was prepared by suspending itazigrel in 0·25% w/v sodium carmellose solution.

Table 1. Composition of aqueous formulations. Saline was added to make the total volume 100 μL.

Formulation	Itazigrel (g)	PS-80 (g)	Oleic acid (g)	Carmellose sodium (g)
1	0·050	0·40	0	0
2	0·250	2·0	0	0
3	0·250	5·0	0	0
4	1·0	20·0	0	0
Solute	0·25	0	0·25	0
Suspension	1·0	0	0	0·25

In-vitro degradation of PS-80 by esterase. Degradation of PS-80 by esterase obtained commercially or from rat intestinal lumen was examined as follows. The esterase solution (1 mg protein mL^{-1}) was prepared by dissolving esterase (obtained commercially) in 0·15 M phosphate buffer (pH 7·0), containing 24 mM taurodeoxycholate; the esterase solution was also used for itazigrel absorption study in the rats. Rat rectal and small intestinal lumenal surfaces were scratched gently using a glass knife to obtain rat intestinal esterase samples, which were suspended in the phosphate buffer containing 24 mM taurodeoxycholate. After centrifugation at 3000 rev min^{-1} for 15 min, supernatant was collected as rat intestinal esterase solution containing 1·5 mg protein mL^{-1}.

Five millilitres of solutions at various PS-80 concentrations (0·4–20% w/v) was mixed with 5·0 mL esterase solution or rat intestinal esterase solution, and then placed in a water bath at 37°C. After 60 min (or 30 min), 1 mL of the solution was collected, and oleic acid, a degradation product of PS-80, was measured with an NFAC-Test Wako assay kit (Wako Pure Chemicals, Osaka, Japan).

Rat study. Male Sprague-Dawley rats, 200–330 g, were fasted for 16 h before the experiment. During the experiment, the rats were intraperitoneally anaesthetized with pentobarbitone (30 mg kg^{-1}). Rectal administration was performed with a cannula inserted from the anus at a depth of 1·5 cm. One millilitre each of the itazigrel formulation and the co-administered solution (the esterase solution, 0·15 M phosphate buffer pH 7·0, or the phosphate buffer containing 24 mM taurodeoxycholate) were mixed before administration. The combinations of formulation and the co-administered solution are described in Table 2. After rectal dosing, the anus was tightened by a thread to avoid leakage. For the intravenous injection, itazigrel

was administered via the rat femoral vein at 250 $\mu g\,kg^{-1}$ (250 μg itazigrel in 1 mL macrogol 200).

Blood samples (100 μL) were collected from the jugular vein at the designated intervals and centrifuged to obtain plasma.

Assay of itazigrel. Before assay, 100 μL methanol was added to 25 μL samples of plasma, and then 50 μL acetonitrile containing 100 ng internal standard (U-64899, 4,5-bis(4-ethoxyphenyl)-2-(trifluoromethyl)-thiazole) was added by mixing vigorously for 30 s. After centrifugation at 3000 rev min^{-1} at 4°C for 30 min, supernatant was collected for assay. Itazigrel was assayed by high-performance liquid chromatography (HPLC), according to the method reported previously (Nishihata et al 1993).

Results and discussion

In-vitro PS-80 degradation by esterase. We investigated the in-vitro degradation of PS-80 by esterase obtained commercially for 60 min, using various concentrations of PS-80. As the concentration of PS-80 increased, a lower degradation was observed (Fig. 1A). Rat small intestinal esterase solution also degraded 1% PS-80 (74·5 ± 5·9 % (n = 3) at 30 min; 98·5 ± 2·7% (n = 3) at 60 min). However, rat rectal esterase solution showed no detectable degradation of 1% PS-80 even at 60 min, and as esterase activity against PS-80 could be ignored in the rat rectal compartment, it was concluded that selection of the rat rectal compartment for the investigation of effect of PS-80 on the itazigrel absorption was reasonable.

Rectal absorption of itazigrel without co-administration of esterase. When Formulation SU (suspension) was administered rectally at a dose of 10 mg kg^{-1} (Table 2), the observed plasma itazigrel concentration was very low (Fig. 2A) and the plasma maximum concentration was about 10 ng mL^{-1} (0·001% dose mL^{-1}) at 60 min. Fig. 2 shows the plasma itazigrel concentrations with percentages of plasma concentration against the doses (% dose mL^{-1}), and Table 2 also shows the ratio of area under the curve (AUC) of plasma itazigrel concentration against the dose. The ratio (AUC)/(dose) after rectal administration of the suspension was 0·15, which is about 3% of the ratio obtained after intravenous administration of itazigrel.

To investigate the effect of PS-80 formulation itself on rectal itazigrel absorption, we administered formulations 1 or 4 with phosphate buffer. In comparison with the dosing of a suspension, significant increases in the values of % dose mL^{-1} of itazigrel in plasma and the AUC/dose were observed (Table 2). However, it was considered that an increase in the concentration of PS-80 in the vehicle caused the slower increase in plasma itazigrel concentration (Fig. 2).

The effects of micelle solubilization on intestinal absorption of solutes have been documented (Westergaard & Dietschy 1976; Amidon et al 1982). It has been reported that rat intestinal absorption of salicylic acid was decreased by the presence of polysorbate esters (Levy & Reuning 1964), whereas intestinal absorption of indoxole was increased when administered with polysorbate esters (Wagner et al 1966). It was considered that the decrease in intestinal salicylic acid absorption is due to the entrapped salicylic acid in the polysorbate ester micelle. On the other hand, it was estimated that the increase in the intestinal absorption of indoxole by the presence of polysorbate esters was because of the enhanced solubilization of indoxole by polysorbate esters as well as the breaking down of the diffusion layer resistance (Westergaard & Dietschy 1976). In the present study, rat rectal absorption of itazigrel increased significantly by incorporating itazigrel in the PS-80 micelle. The PS-80 micelle may have supplied the maximal solution (itazigrel) concentration to the rectal epithelial membrane. An entrapping effect of itazigrel in PS-80 micelle in formulation 4 is also probable, since we observed slower itazigrel absorption after administration of formulation 4 with a high PS-80 concentration.

Rectal absorption of itazigrel with co-administration of esterase. We investigated the effect of esterase co-administered on rectal absorption of itazigrel administered in PS-80 micelle formulation by changing the concentration of PS-80. Fig. 2C shows the value of % dose mL^{-1} of itazigrel in plasma after the dosing. When formulation 1 was co-administered with the esterase solution (Fig. 2C), an increase in the value of % dose mL^{-1} for itazigrel was observed in comparison with that when administered with the phosphate buffer alone (Fig. 2B). The AUC/dose value obtained was 7·88, which was very similar to the value after intravenous injection and was about 1·7 times the value without esterase. Thus, the co-administration of the esterase solution increased the rectal absorption of itazigrel when administered in vehicle containing PS-80. When esterase solution was co-administered with the suspension, no significant effect was observed (data not shown).

The more diluted the PS-80 micelle, the more rapid is the increase in the value of % dose mL^{-1} itazigrel in plasma

Table 2. Absorption (AUC/dose) of itazigrel after rat rectal administration for nearly 180 min after administration.

Formulation	Solution[a] co-administered	Itazigrel (mg kg^{-1})	AUC_{0-180} (mg min L^{-1})	AUC/dose (min L^{-1})
Rectal administration:				
Suspension	Buffer	10·0	2·01 ± 0·48	0·20 ± 0·05[a]
	Buffer	0·5	2·38 ± 0·46	4·75 ± 0·91[a,b]
	Buffer	10·0	22·32 ± 2·29	2·23 ± 0·23[a,b,c]
	Esterase	0·5	3·94 ± 0·31	7·88 ± 0·62[a,c]
	Esterase	2·5	14·43 ± 1·42	5·77 ± 0·57[a]
	Esterase	2·5	13·36 ± 1·39	5·34 ± 0·56[a]
	Esterase	10·0	34·54 ± 7·93	3·45 ± 0·79[a,d]
	Taurodeoxycholate	0·5	0·80 ± 0·01	1·61 ± 0·16[c,d]
	Buffer	10·0	1·20 ± 0·12	0·12 ± 0·01
Intravenous administration:		0·25	1·72 ± 0·01	6·89 ± 0·35

[a] $P < 0·01$ compared with suspension, [b] $P < 0·01$ compared with intravenous administration, [c] $P < 0·01$ compared with 0·5 mg kg^{-1} in buffer, [d] $P < 0·01$ compared with 0·5 mg kg^{-1} with esterase.

(Fig. 2C). However, the AUC/dose value for formulation 2 was about 5·8 which was similar to the value for formulation 3 (5·3), and was about 80% of the value after intravenous injection (Table 2). The slowest increase in value of % dose mL^{-1} of itazigrel in plasma and the lowest AUC/dose value were observed for formulation 4 which contained a high concentration of PS-80.

To investigate the effect of taurodeoxycholate contained in the esterase solution on the itazigrel absorption, we co-administered phosphate buffer containing taurodeoxycholate with formulation 1, and observed a suppressing effect (Fig. 2B and Table 2). Further, the increase in itazigrel absorption administered in PS-80 vehicles in the presence of esterase is not due to oleic acid degradation product of PS-80, since oleic acid itself did not increase the itazigrel absorption (Table 2).

When PS-80 micelle containing itazigrel was co-administered with esterase solution, further enhanced rectal absorption of itazigrel was observed in comparison with the results obtained without esterase (Table 2). As the concentration of PS-80 decreased, PS-80 degraded more in the presence of esterase (in-vitro, Fig. 1A) and the AUC/dose value of itazigrel increased (in-vivo, Table 2). A good linear relationship was also observed between the in-vitro degradation ratio of PS-80 and the in-vivo AUC/dose value of itazigrel (Fig. 1B). Thus, it is proposed that PS-80 micelle as a solubilizer plays a role in delivering itazigrel effectively to the absorption site, and that the biodegradable characteristics of PS-80 release itazigrel effectively for absorption by eliminating the micelle-entrapped drug when it encounters esterase.

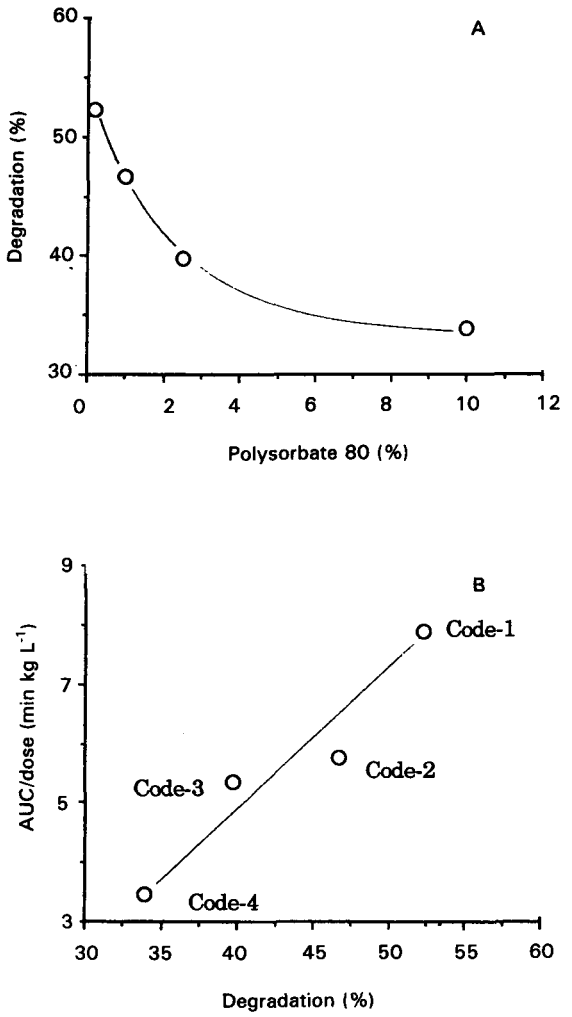

FIG. 1. A. In-vitro degradation of polysorbate 80 incubated in the presence of esterase at 37°C for 60 min. Each value represents the mean ± s.d. (n = 3). B. Relationship between the bioavailability of itazigrel after rectal administration of the formulations and the in-vitro degradation of PS-80 by esterase. The number represents the formulation described in Table 1.

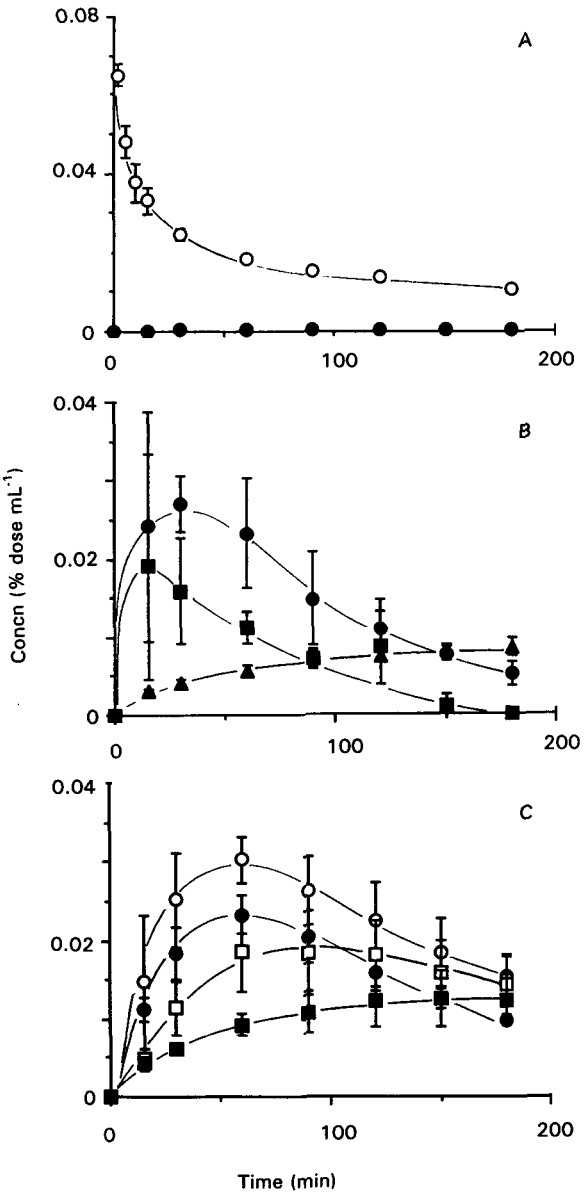

FIG. 2. Plasma itazigrel concentration (% dose mL^{-1}) profiles following administration of itazigrel. A. Intravenous administration at a dose of 250 $\mu g\,kg^{-1}$ (○) and after rectal administration of itazigrel suspension (●) at a dose of 10 $mg\,kg^{-1}$. B. After rectal administration of formulations co-administered with the phosphate buffer (● formulation 1; ▲ formulation 4) or with the phosphate buffer containing taurodeoxycholate (■ formulation 1). C. After rectal administration of the formulations containing polysorbate 80 coadministered with esterase solution. ○ formulation 1 (0·5 $mg\,kg^{-1}$), ● formulation 2 (2·5 $mg\,kg^{-1}$), □ formulation 3 (2·5 $mg\,kg^{-1}$), ■ formulation 4 (10 $mg\,kg^{-1}$). Each value represents the mean ± s.d. (n = 4).

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Pharmacokinetics and brain distribution of zolpidem in the rat after acute and chronic administration

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Abstract—The pharmacokinetics of zolpidem were studied after single dose, administered for either 7 or 28 days to rats. Thirty minutes after the last dose, animals were killed and the brain removed. The highest concentrations in plasma, which were observed at the first sampling time (0·5 h) were 2341 ± 540 (day 0), 1956 ± 325 (day 7) and 2908 ± 1369 ng mL^{-1} (day 28). Corresponding AUC values of 1742 ± 488, 1583 ± 422 and 2683 ± 1249 ng mL^{-1} h were found. MRT increased significantly from 0·46 ± 0·06 h on day 0 to 0·67 ± 0·02 h on day 28. The cerebral levels showed no significant change during the chronic administration (766 ± 285, 685 ± 171 and 887 ± 264 ng g^{-1}, respectively). No modification of the principal kinetic parameters was detected up to the 28th day of treatment.

In recent years, zolpidem (Fig. 1), a novel non-benzodiazepine hypnotic agent belonging to a new chemical series, the imidazopyridines, has been developed and used clinically (Arbilla et al 1985; Depoortere et al 1986; Benavides et al 1988). The activity of benzodiazepines is mediated by their interaction with central ω (BZD) modulatory sites associated with the $GABA_A$-receptor complex (Bosmann et al 1978; Braestrup & Nielsen 1981). The preferential affinity of zolpidem is for the ω_1 site of the global GABA receptor (Langer & Arbilla 1988; Benavides et al 1988), but the most recent results suggested that zolpidem sites are associated to at least three subtypes of receptors (Benavides et al 1993).

In separate clinical studies, zolpidem was generally well tolerated (Palminteri & Narbonne 1988; Licciardello & Licini 1992). Zolpidem did not cause signs of withdrawal symptoms or tolerance (Schlisch et al 1991; Maarek et al 1992) with chronic treatment. A careful use and study of zolpidem in the treatment of insomnia is needed to clarify this trend and in particular to compare zolpidem with benzodiazepines in a widespread double-blind study.

Pharmacokinetic factors may be one of the elements of the tolerance. Time-limited pharmacokinetic properties of zolpidem for no more than two weeks have been published in various species (Thénot et al 1988; Langtry & Benfield 1990), and recently for twenty-one days in haemodialysed uraemic patients (Fillastre et al 1993), although, the treatment is usually for at least one month. Chronic administration of zolpidem does not modify its absorption rate (Durand et al 1992), but a modification of its clearance is possible.

The present study was carried out to ascertain the plasma pharmacokinetic parameters after acute, subchronic (7 days) and chronic (28 days) intraperitoneal administration in the rat. A brain distribution study complemented the pharmacokinetic findings for plasma.

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Materials and methods

Animals. Male Sprague-Dawley rats, 400–450 g (Depré, France), were housed four per cage with free access to food and water under a 12-h light/12-h dark cycle. The animals were acclimatized for one week before the start of the experiments.

Study design. In the acute experiment, rats (five per group) received a single intraperitoneal injection of zolpidem (6·22 mg kg^{-1} as the hemitartrate salt corresponding to 5 mg kg^{-1} of the free base), which was administered dissolved in physiological saline solution (0·25 mL/100 g). Zolpidem was a gift from Synthelabo (Paris, France). In the chronic experiments, rats received early morning injections of 5 mg kg^{-1} zolpidem (at the same hour) for 7 days or for 28 days.

Venous blood samples were taken from the tail into tubes containing lithium heparin, at 0·5, 1, 1·5, 2, 3, 4, 6, 8, 10, and 24 h after administration. Plasma was quickly separated and stored

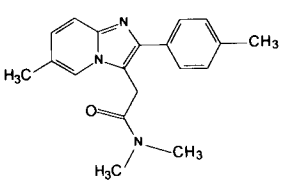

FIG. 1. Chemical structure of zolpidem.