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Absorption of Itraconazole from Rat Small Intestine

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이트라코나졸의 랫트 소장으로부터의 흡수

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The absorption characteristics of itraconazole, which is an antifungal agent, from intestinal segments in the anesthetized rat *in situ* were investigated in order to design an effective oral drug delivery system. The pH-solubility profile of itraconazole, the rate and extent of absorption of itraconazole, the optimal absorption site(s) of itraconazole and the absorption enhancing effect of sodium cholate on itraconazole were examined in the present study. *In situ* single-pass perfusion method and recirculating perfusion technique using duodenum(D), jejunum(J) and ileum(I) were employed for the calculation of apparent permeability(Pe) and apparent first-order rate constant(Kobs), respectively. The results of this study were as follows: (1) Itraconazole showed appreciable aqueous solubility only at pH values of below 2.0. (2) pe(cm/sec) decreased in the following order: D($10.24\pm 1.78\times 10^{-4}$)>J($8.86\pm 0.79\times 10^{-4}$)>I($3.78\pm 0.13\times 10^{-4}$). (3) Kobs($min^{-1}$) decreased in the following order: J($17.12\pm 3.19\times 10^{-3}$)>D($13.37\pm 0.6\times 10^{-3}$)>I($11.05\pm 0.91\times 10^{-3}$). (4) The solubility of itraconazole markedly increased with the increase of the concentration of sodium cholate. (5) The addition of 10 mM sodium cholate significantly increased the apparent first-order rate constant of itraconazole in the ileum by a factor of 6.8.

Keywords—itraconazole absorption, solubility, small intestine, absorption promoter.

Progressive improvement has been achieved in antifungal chemotherapy in recent years. Among azole compounds, miconazole was introduced for use in the management of dermatology and gynaecology of the first topical agent in 1971.[1] Although many new azol and triazole derivatives have been synthesized and used in antifungal therapy, but only itraconazole has been successful in oral treatment of superficial and deep mycoses in humans. Its chemical structure is given in Fig. 1.

Itraconazole is the prototype of a class of triazole antifungal agent with high lipophilicity, good oral absorption and extensive tissue distribution.[2,3] The drug has been shown to be highly effective in common fungal infections such as oral[4] and vaginal[5-7] candidiasis, pityriasis versicolor,[7-11] and skin dermatophytosis.[7,10,12] In addition, excellent antifungal activities have been obtained in patients with systemic mycoses,[13-15] or in immunosuppressed patients with HIV-infections.[16] Van Peer *et al.* reported that itraconazole should be administered immediately after a meal or with a meal to ensure optimal oral systemic availability.[17]

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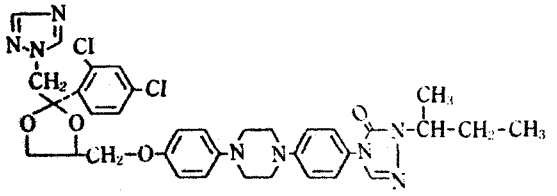

Chemical name: (±)-2-sec-Butyl-4-[4-(4-[(2R, 4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-ylmethoxy] phenyl-piperazin-1-yl)phenyl]-2, 4-dihydro-1,2,4-triazol-3-one.

Figure 1—Structural formula of itraconazole.

Up to date, the underlying causes of absolute bioavailability (55%)[18] and the difference of relative bioavailability in fasting and in fed[19] of itraconazole have not been clearly established. Based on the above reasons, this study was initially to examine the absorption kinetics (rate and extent), secondly to identify an optimal site(s) for absorption of itraconazole in three different small intestinal segments of rat e.g., duodenum(D), jejunum (J) and ileum(I) and finally to evaluate the absorption enhancing effect of sodium cholate on itraconazole in the rat ileum.

Experimental

Reagents

Itraconazole(Lot:00074745) was supplied by Janssen Pharmaceutica(Beerse, Belsium). Sodium cholate was purchased from Sigma Chemical Company(St. Louis, MO). All other chemicals were of pharmacopoeial or HPLC grade.

Apparatus

The instruments employed were HPLC(Gilson Model 305 Pump) and UV/VIS Spectrophotometer (Shimadzu UV-240). A peristaltic pump (model-1303P2 Korea Manhattan Co.) was used to perfuse the solution in *in situ* perfusion technique.

Animals

Male Sprague-Dawley rats weighing 200 to 310g were fasted for 12–16 hrs prior to initiation of experiment, but water was available *ad libitum*.

Determination of the Solubility of Itraconazole

To obtain the pH-solubility profile of itraconazole which is very poorly water soluble(<0.0001g/100 m*l* in water, pH 6.7), the solubility test of itraconazole in the different pH(1.2~8.0) buffer solutions was conducted. The solubilities of itraconazole in phosphate buffer solution(pH 6.5) were examined by increasing the concentration of sodium cholate. For this experiment, an excess of drug was equilibrated with pH buffer solutions (pH 1.2, 2.0, 2.3, 3.0, 5.6, 7.0, 8.0) at 23℃ by sonication and shaking for more than 6 hrs. The solubilities of the compound in phosphate buffer solution(pH 6.5) in proportion as concentration increase of sodium cholate were examined by equilibrating an excess of itraconazole for more than 6 hrs with 5 m*l* of each medium. The solutions were then filtered through a 0.45-μm Millipore filter and the drug concentration of a suitable aliquot was analyzed. The aliquots were analyzed at the wavelength of 264 nm with a spectrophotometer. Identical dilutions of drug-free media or solvents served as blank.

In Situ Absorption Studies

The experimental technique was adapted from that of Farraj *et al.*[20] for perfusion of the rat small intestine. Three intestinal segments used in this study were defined as duodenum(pyloric sphincter to the ligament of Treitz), jejunum(the next 10 cm portion of the tract following the ligament of Treitz), and ileum(the next 10 cm ended at ileocecal junction). To prepare the drug solution in 2% PEG 400, 10 mg of itraconazole powder was added to the 10 m*l* of PEG 400 and sufficient isotonic phosphate buffer solution was added to a total volume of 500 m*l*. The final drug concentration was 20 μg/m*l*. A nonabsorbable marker of the perfusate was not used in this study. Water absorption was not significant (±2%)[21] because this study was examined relatively short length of each intestinal segment. The solution was maintained at 37℃ and was perfused with a peristaltic pump at a rate of 0.6 m*l* per minute.[22] To determine the rate and extent of absorption, two different *in situ* perfusion techniques, e.g., recirculating perfusion, single-pass perfusion, were used in this study.

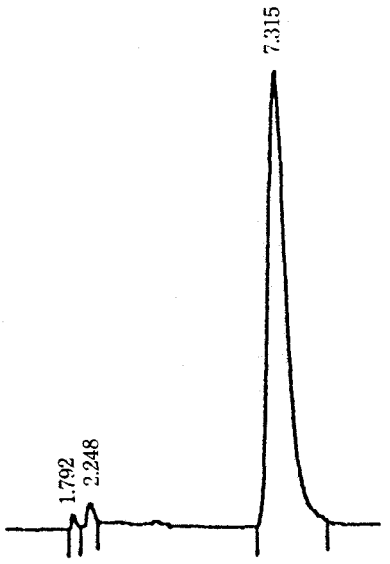

Figure 2—Typical chromatogram of itraconazole.

(a) *In Situ* Single-Pass Perfusion Method—After initial 5 min. of lag time, drug samples were collected from the segmental outflow cannula at 15 min. intervals up to 120 min.. The concentration of drug was measured in each of the collected samples.

(b) *In Situ* Recirculating Perfusion Technique—The tubings attached to the end outflow cannula were transferred to a beaker containing 10 m*l* of drug solution and then continuously circulated through the intestinal segment for 2 hrs. After initial 5 min. of lag time, drug samples were obtained by removing 0.2 m*l* of the perfusate at 15 min. intervals up to 120 min..

Analytical Procedure

The remaining concentrations of itraconazole in the intestinal perfusate were quantitated by reverse phase HPLC system method(Fig. 2). The HPLC procedure described in this paper was based upon that of Woestenborghs, *et al.*[23] The mobile phase was composed of water: acetonitrile(20 : 80) supplemented with diethylamine at a concentration of 300 μ*l*/*l*. The pH was adjusted to 7.8 with orthophosphoric acid. The eluent was filtered and degassed under reduced pressure before use and was pumped at a rate of 1.0 m*l* per min.

Investigation of Itraconazole Ileal Absorption

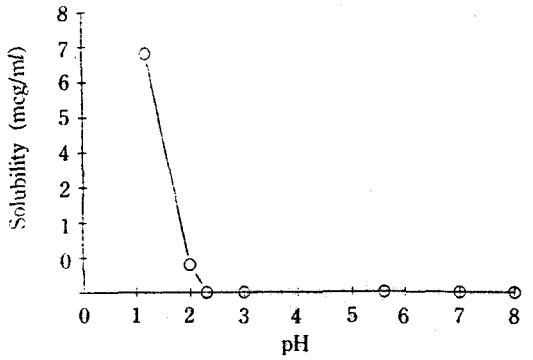

Figure 3—pH-solubility profile of itraconazole.

Enhancement

As reported earlier, we investigated the enhancement of ileal absorption by using 10 mM sodium cholate because of the lower Kobs($11.05 \pm 0.91 \times 10^{-3}$ min^{-1}) of itraconazole in the ileum of rat. The technique used in this study was an *in situ* recirculating technique which exaggerated the decrease in drug concentration. The perfusate was prepared by 10 mM sodium cholate being added to a concentration of 20 μg/m*l* of phosphate buffer drug solution. Ten milliliters of the solution was then perfused into the ileum of rat for 2 hrs. The concentration of itraconazole remaining in the ileal perfusate was measured quantitatively by reverse phase HPLC using the conditions described in the analytical method.

Results and Discussion

pH-Solubility Profile

The drug, which exists in very fine powder and metastable liquid forms, had a pKa value of 3.7 (extremely weak base) and a very poor solubility of less than 0.0001g/100 m*l* in water(pH 6.7) as reported earlier.[24] It had apprecible aqueous solubility only at pH values of less than 2.0(Fig. 3). Fig. 4 showed the solubility profile of itraconazole in isotonic phosphate buffer solution(pH 6.5) containing sodium cholate of various concentration at 37℃. As compared with the solubility of practically zero in this pH, the solubility of itraconazole markedly increased with the increase of the concentration of sodium cholate.

Absorption Characteristics in Small Intestinal

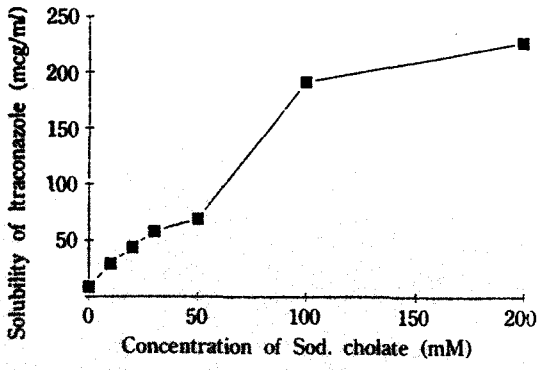

Figure 4—The effect of sodium cholate on solubility of itraconazole in phosphate buffer solution (pH 6.5) at 37°C.

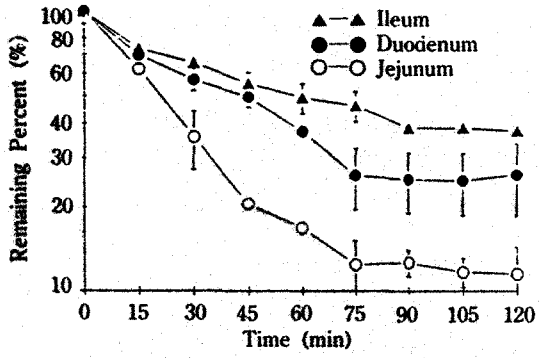

Figure 5—Semilog plots of the percentage of itraconazole remaining versus time in three intestinal segments of rat by using an *in situ* single-pass perfusion method. Each value is MEAN± S.E. (n=3)

Table I—*Apparent Permeabilities and Percent Absorbed of Itraconazole after 2 hrs in Three Intestinal Segments of Rat by Using an in Situ Single-pass Perfusion Method.*

Intestinal Segment	Intestinal Radius (cm)	Intestinal Length (cm)	Percent Absorbed (%)	Apparent Permeability (cm/sec×10⁴)
Duodenum	0.275	8	73.80	10.24
		(1.41)	(2.73)	(1.78)
Jejunum	0.45	9.5	88.50	8.86
		(1.08)	(2.90)	(0.79)
Ileum	0.4	10.3	62.47	3.78*
		(0.24)	(1.02)	(0.13)

(Numbers in parentheses denote standard errors (n=3))

*$p<0.05$

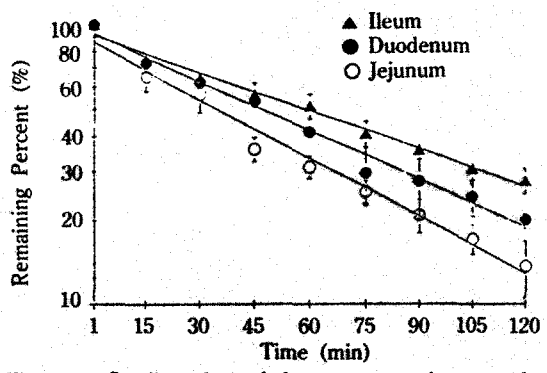

Figure 6—Semilog plots of the percentage itraconazole remaining versus time in three intestinal segments of rat by using an *in situ* recirculating perfusion technique. Each value is MEAN± S.E. (n=3)

Segments

(a) Apparent Permeability—The percentage of itraconazole remaining in three segments of the rat intestine as a function of time are shown in Fig. 5.

In this study, the apparent permeabilities per unit length were calculated by using *in situ* single-pass perfusion method in order to compare the intrinsic absorptivity of drug in three intestinal segments of rat. The fraction of drug remaining to be absorbed at steady state within a specified intestinal lumen taken as a cylinder is described by the equation of Higuchi and Ho.[25];

$$C(l)/C(0)=\exp[-2\pi rlPe/Q] \quad (1)$$

in which C(0) is the drug concentration entering the intestinal segment; C(l) is the drug concentration leaving the intestinal segment at time, t; l is the intestinal length of each segment in cm; r is the effective radius of the intestinal lumen in cm; Q is the bulk fluid flow rate through the segment in cm^3/sec; and Pe is the apparent permeability coefficient in cm/sec.

The fraction absorbed (Fa) up to a specific time point was then calculated as;

$$Fa=1-C(l)/C(0) \quad (2)$$

Substituting equation (1) into equation (2), rearranging and taking natural logarithm results in the expression;

$$Pe=-Q/2\pi rl\times\ln(1-Fa) \quad (3)$$

The apparent permeabilities of itraconazole in three intestinal segments of rat were calculated

Table II—*Apparent First-Order Rate Constants and Remaining Percentage of Itraconazole after 2 hrs in Three Intestinal Segments of Rat by Using an in Situ Recirculating Perfusion Technique.*

Intestinal Segment	Intestinal Radius (cm)	Intestinal Length (cm)	pemaining Percent (%)	Apparent First-Order Rate Constant ($min^{-1} \times 10^{3}$)
Duodenum	0.275	9.3	20.15	13.37
		(0.94)	(1.45)	(0.60)
Jejunum	0.45	9.3	13.70	17.12
		(0.24)	(4.48)	(3.19)
Ileum	0.4	9.4	26.70	11.05
		(0.66)	(2.90)	(0.91)

(Numbers in parentheses denote standard errors (n=3)) *p<0.05

Table III—*Comparison of Absorption Promoting Effect with Different Adjuvants after 1 hr in the Ileum of Rat by Using an in Situ Recirculating Perfusion Technique.*

Adjuvant	Intestinal Radius (cm)	Intestinal Length (cm)	Remaining Percent (%)	Apparent First-Order Rate Constant ($min^{-1} \times 10^{3}$)
REG 400	0.4	9.4	51.27	11.30
		(0.66)	(7.38)	(2.30)
Sodium Cholate	0.4	9.25	1.01	76.75*
		(0.25)	(2.45)	(1.52)

(Numbers in parentheses denote standard errors (n=3)) *p<0.05

using Eq.3 with effective radius of intestine[26] and shown in Table I.

(b) Apparent First-Order Rate Constant—An *in situ* recirculating perfusion technique was used to determine the rate of absorption of drug in three intestinal segments of rat. As shown in Fig. 6, the remaining concentration of itraconazole for 2 hrs followed a first order kinetics. The time dependence of the luminal concentration, C can thus be written as;

$$\ln C(l)/C(0) = -Kobs \times t \quad (4)$$

The Kobs of itraconazole in three intestinal segments of rat calculated by plotting percent remaining of itraconazole [C(*l*)/C(0)×100] versus time on semilog paper according to Eq.4.(Table II)

The Effect of Adjuvants on the Ileal Absorption

Relatively low concentrations of some bile salts, e.g. 0.2% of sodium taurocholate or sodium cholate did not affect small intestinal mucosal structure.[27] And light and electron microscopic studies did not reveal any epithelial damage on enhancement of

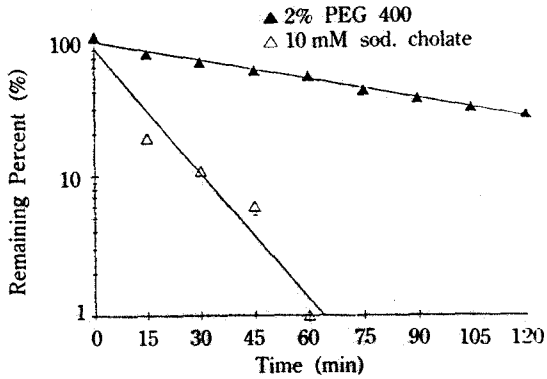

Figure 7—The effect of adjuvants on the absorption of itraconazole in the ileum of rat by using an *in situ* recirculating perfusion technique.

ileal insulin absorption by 1%(w/v) of sodium cholate.[28] Fig. 7 showed that the effect of 10 mM sodium cholate on the ileal absorption was significantly greater than 2% PEG 400 which was used *in situ* previously. In accordance with the results in Table III, sodium cholate of low concentration increased the ileal absorption in comparison with

PEG 400 by a factor of 6.8.

Conclusions

In situ perfusion techniques were employed to examine the absorption behaviour of itraconazole which is very poorly water soluble(<0.0001g/100 ml in water, pH 6.7) and ileal absorption enhancement of itraconazole by sodium cholate was also investigated. Summarizing the present studies, it can be concluded as follows:

1. The solubilities of itraconazole, which had appreciable aqueous solubility only at the pH below 2.0, markedly increased with increasing in the concentration of sodium cholate.

The results showed that sodium cholate may significantly increase the solubility and the bioavailability of itraconazole, of which solubility would likely be absorption rate limiting factor due to its high lipophilicity (partition coefficient in the n-octanol/water, logP=5.66).

2. As a result of being obtained after *in situ* single-pass perfusion method and recirculating perfusion technique, respectively, the decreasing order of Pe(cm/sec) was D($10.24\pm 1.78\times10^{-4}$)>J($8.86\pm 0.79\times10^{-4}$)>I($3.78\pm 0.13\times10^{-4}$) and that of Kobs ($min^{-1}$) was J($17.12\pm 3.19\times10^{-3}$)>D($13.37\pm 0.6\times10^{-3}$)>I($11.05\pm 0.91\times10^{-3}$).

Concerning these results, two possibilities could be considered. One is that intrinsic absorptivity would be greater in the upper small intestine than in the lower small intestine because Pe values in the D and J were similar decrease followed by the I These phenomena is the reason why the difference in surface area for absorption due to the lower concentration of villi and microvilli in the lower portion of small intestine.[29]

The other possibility is that absorption occurs throughout the small intestine and there is no site-limitation because there is no significant difference in Kobs in J, D and I. Therefore half-life of itraconazole may be long *in vivo*. A similar phenomenon has been reported in absorption experiment with other drugs, e.g. antifungal drug griseofulvin.[30,31] In the case of highly lipophilic drugs with very low aqueous solubility, the overall absorption process is slow because of limiting solubility in gastrointestinal fluid and then incomplete bioavailability arises.

It is conclusive from the above discussion that the increased absorption will result from increased solubility of itraconazole in bile and delayed gastric emptying time.

3. Sodium cholate used to enhance the absorption of itraconazole in ileal segment of rat increased by a factor of 6.8, comparing with PEG 400.

From the above result, one may conclude that relatively low concentration of 10 mM sodium cholate can enhance drug uptake to a significant extent. But in order to apply the compound as safe absorption promoter in man, the phenomena correlated with its effect on drug uptake including mucosa damage should be observed if necessary.

Further studies will be therefore required to fully elucidate the absorption mechanism of itraconazole.

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