Pharmaceutical Research, Vol. 22, No. 3, March 2005 (© 2005)
DOI: 10.1007/s11095-004-1879-6

Research Paper

Relationship Between Loperamide-Induced Sedative Effect and Digoxin Pharmacokinetics in Healthy Japanese Subjects

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Received August 1, 2004; accepted December 9, 2004

Purpose. Loperamide-induced suppressive effects on central nervous system closely relate to a lack of or decline in the P-glycoprotein (P-gp) function. The aim of this study was to determine the loperamide-induced sedative effect quantitatively and to investigate possible alterations in the pharmacokinetics of digoxin, a substrate for P-gp, in Japanese subjects.
Methods. Loperamide hydrochloride (2 mg) was administered orally to 26 subjects and the critical flicker-fusion frequency threshold (CFF) values were measured every 30 min separately by portable instrument. Further, digoxin (0.25 mg) was administered to 8 subjects, and the plasma concentration was determined.
Results. In five subjects who complained of drowsiness, the CFF values more remarkably decreased compared with those in the other subjects. The T_{max} and mean residence time (MRT) values of digoxin pharmacokinetics in four subjects with drowsiness were significantly lower and C_{max} was higher than those in four subjects with marginal effect. Moreover, there were good correlations between the CFF value-time profile and the C_{max}, T_{max}, and MRT of digoxin.
Conclusions. The determination of the CFF value after oral administration of loperamide will be useful for evaluating varied P-gp function and for anticipating individual variations in the disposition of P-gp substrates in humans.

KEY WORDS: digoxin; individual variations; loperamide; P-glycoprotein; polymorphism.

INTRODUCTION

It is well-known that interpatient variations in drug disposition occasionally cause unexpected increases or decreases in the pharmacological effect of or adverse reactions to certain drugs. Recently, genetic polymorphism has been hypothesized as one of the reasons for this interpatient variation. In particular, genetic polymorphisms in transporters are responsible for variations in intestinal absorption, renal excretion, and distribution to organs of drugs in patients (1–3).

Loperamide is an antidiarrhetic, which reduces intestinal motility by its action on opiate receptors in the intestine (4). Although loperamide is an agonist of opiate receptors, it has little effect on the central nervous system (CNS) at normal dosages (5) as loperamide is a substrate of P-glycoprotein (P-gp) and its penetration into the brain is potently restricted by P-gp (6). However, drowsiness or other central opiate effects occasionally occur in patients as reported in Post Marketing Surveillance of Lopemin in Japan. It seems that loperamide-induced effects on the CNS are associated with the expression and/or function level of P-gp in the blood-brain barrier. Sadeque *et al.* (7) reported that the measurement of respiratory depression after oral administration of loperamide was useful in evaluating loperamide-induced effects on the CNS, but the procedure was complicated. On the other hand, the measurement of critical flicker-fusion frequency threshold (CFF) to evaluate the suppressive effects on the CNS is simple and easy. CFF is the lowest frequency of flickering light (measured in Hz) that is required to produce an appearance of steady light to an observer. When a light is flickered at rates equal to the CFF, the individual flashes cannot be resolved and the light is indistinguishable from a steady, nonflickering light. This method has been used to evaluate numerically the effect on the CNS of the administration of antihistamines (8), benzodiazepines (9,10), and antipsychotics (11).

P-gp is the ATP-dependent pump that exports the sub-

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ABBREVIATIONS: AUC, area under the digoxin concentration curve; C_{max}, maximum plasma concentration; CFF, critical flicker-fusion frequency threshold; CL_{tot}/F, total clearance divided by bioavailability; CNS, central nervous system; CYP, cytochrome P-450; ΔCFF-AUC, area under the ΔCFF curve; Ka, absorption constant; Ke, elimination constant; MRT, mean residence time; P-gp, P-glycoprotein; T_{max}, time at maximum plasma concentration.

strate from the inside to the outside of the cells (12), and it has been found not only in the brain but also in various organs such as the intestine, liver, and kidney. It is well-known that P-gp recognizes many kinds of exogenous and endogenous substances (e.g., antitumor drugs, steroid hormones, and cyclosporin A). Digoxin, which is used in the treatment of congestive heart failure, is a substrate for P-gp, and its intestinal absorption and renal excretion is thought to be regulated by P-gp (13,14). Recently, many groups have reported on the relationship between the pharmacokinetics of digoxin and the genetic polymorphism of P-gp (15); however, there is some disagreement in their results. Namely, Hoffmeyer *et al.* (16), Johne *et al.* (17), Kurata *et al.* (18), and Verstuyft *et al.* (19) reported that the blood concentration of digoxin after oral administration was higher in the subjects harboring a mutant allele (C3435T) at exon 26 of the P-gp gene, but Sakaeda and co-workers reported opposite results (20,21). Furthermore, Gerloff *et al.* reported that the P-gp genotypes did not influence the absorption of digoxin in healthy subject (22). Thus, the estimation of digoxin (and other P-gp substrates) disposition in order to make use of P-gp polymorphism is difficult at present.

There have been few reports on the comparison of drug dispositions using two or more substrates for P-gp in the same subjects to evaluate the function of P-gp. It can be considered that the suppressive effects on CNS after loperamide administration would be related to the digoxin disposition, especially its intestinal absorption in humans, because the metabolism of digoxin is limited and its absorption from the intestine is regulated to a large degree by P-gp. In this study, we quantitatively determined the sedative effect induced by loperamide in healthy Japanese subjects by the measuring CFF value and compared the pharmacokinetics of digoxin between in sedative and nonsedative subjects.

MATERIALS AND METHODS

Materials

Lopemin capsules (1 mg of loperamide HCl) and Digosin tablets (0.25 mg of digoxin) were obtained from Dainippon Pharmaceuticals (Osaka, Japan) and Chugai Pharmaceuticals (Osaka, Japan), respectively. All other reagents were of analytical grade or higher.

Evaluation of Sedative Effect after Loperamide Administration in Healthy Subjects

Informed consent was obtained from each subject after explaining the aim and potential risk of this study, which was approved by the local committee of the Health Sciences University of Hokkaido. Fifteen healthy male and 11 female subjects [age: 27.9 ± 8.8 years (mean ± SD), weight: 60.0 ± 8.4 kg] freely consented to participate in the current study. After overnight fasting, the subjects received 2 mg of loperamide HCl (2 capsules of Lopemin) with 200 ml of water at 9:30 am. The CFF value for each subject was determined at 0, 30, 60, 90, 120, 150, and 180 min after administration using a portable JM101 instrument (Jimbo Engineering, Tokyo, Japan). At the beginning of the test, a red light-emitting diode was set to flicker at 50 Hz, at which frequency the light could not be identified as flickering by observer. The flickering cycle was then automatically and gradually decreased to 20 Hz, and observer stopped the JM101 apparatus when he or she could identify the flickering. This flickering cycle (Hz) was taken as the CFF value. The CFF values were determined five times at each time point and the mean was calculated. The coefficient of variance of the CFF value at each time was less than 5%. The ΔCFF was calculated by subtracting the CFF value at each time from the CFF value at time zero.

Digoxin Plasma Concentration in Healthy Subjects

We tentatively defined the subjects with no symptoms as group 1 and subjects with moderate or strong drowsiness as group 2 in the loperamide study. Eight healthy subjects (4 subjects from each group, 7 male and 1 female, age: 31.9 ± 11.8 years, weight: 59.3 ± 4.9kg) were re-informed about the object and possible risk of this study, and consent was re-obtained. They were administered 0.25 mg digoxin (a tablet of Digosin) with 200 ml of water after overnight fasting. This study was performed more than 2 weeks after loperamide administration. About 5 ml of blood was collected at 0, 0.5, 1, 2, 4, and 7 h after administration using a heparinized syringe. Blood samples were centrifuged immediately at 3000 × g for 15 min, and the separated plasma was stored at –20°C until analysis.

Analytical Procedure

The concentration of digoxin in the plasma was measured by fluorescence polarization immunoassay using a TDx analyzer (Abbott Laboratories, Abbott Park, IL, USA). The lower limit of quantification was 0.2 ng/ml. The interassay coefficients of variation at concentrations of 0.75 ng/ml, 1.50 ng/ml, and 3.50 ng/ml were 8.13%, 7.60%, and 4.57%, respectively.

The pharmacokinetic parameters such as absorption constant (Ka), elimination constant (Ke), and total clearance divided by bioavailability (CL_{tot} /F) of digoxin were calculated by the least squares method as a one-compartment model (23). The mean residence time (MRT) was calculated by moment analysis (24). The maximum concentration (C_{max}) and the time at C_{max} (T_{max}) were obtained from observed data (C_{max}*obs*, T_{max}*obs*) and simulated from the theoretical equation (C_{max}*sim*, T_{max}*sim*). The area under the digoxin concentration curve for 0 to 4 h and 0 to 7 h ($AUC_{0\text{-}4h}$and $AUC_{0\text{-}7h}$, respectively) were calculated by the trapezoidal method.

Unpaired *t* test was used to determine the statistical significance of differences between experimental groups, and a value of $p < 0.05$ was considered significant.

RESULTS

Time-Profiles of ΔCFF in Subjects after Loperamide Administration

Typical time-profiles of CFF values in two subjects after oral administration of loperamide are shown in Fig. 1. It was confirmed that the diurnal variation in CFF values without the medication was less than 1 Hz (data not shown). In subject no. 5, the CFF value fell drastically to a minimum value at 2 h after medication, which was followed by a steady increase. This subject complained of strong drowsiness at 1.5 to 2 h after medication. On the other hand, a similar but less

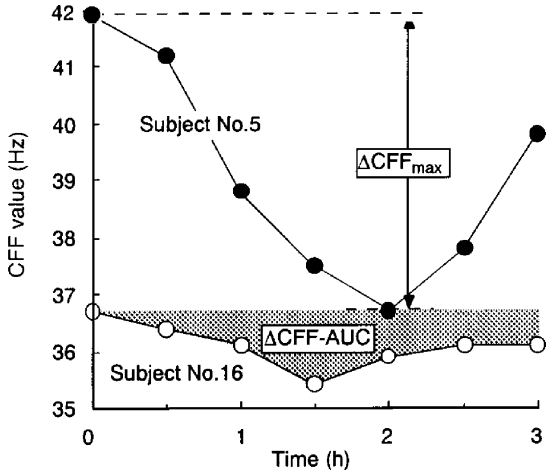

Fig. 1. Typical time courses of CFF values after oral administration of loperamide HCl (2 mg). Two capsules of Lopemin were administered to a fasted subject with 200 ml of water. CFF values were measured by JM101 at each time. ΔCFF_{max} is the difference between CFF value at time zero and the minimum value of CFF. ΔCFF-AUC is the area under the ΔCFF curve calculated by the trapezoidal method.

marked change in CFF values was noted in subject no. 16, who felt no subjective symptoms. Therefore, these results strongly suggested that the CFF values would be decreased by the loperamide dosing irrespective of subjective symptoms. Accordingly, we calculated the difference between CFF value at time zero and the minimum value of CFF as ΔCFF_{max} obtained from 26 subjects. Furthermore, the area under the ΔCFF curve (ΔCFF-AUC) was calculated by the trapezoidal method. Figure 2 shows a linear correlation between ΔCFF-AUC and ΔCFF_{max}. Group 2 comprised the subjects who felt moderate or strong drowsiness in this examination, and their ΔCFF_{max} and ΔCFF-AUC were relatively higher than those of group 1.

Pharmacokinetic Parameters of Digoxin in Group 1 and Group 2 Subjects

In the digoxin study, variations in the plasma concentration of digoxin in four subjects from group 1 and four subjects from group 2 were determined. The plasma concentrations of digoxin in group 2 were comparatively higher than those in group 1 (Fig. 3). The T_{maxobs} value in all subjects of group 2 was at 1 h, whereas it was at 2 h in group 1 subjects, and C_{maxobs}, T_{maxsim}, and MRT values in group 2 were significantly lower than in group 1 (Table I). Ka and $AUC_{0\text{-}4h}$ values tended to be higher in group 2 than in group 1. We also calculated the correlations between these parameters and ΔCFF_{max} or ΔCFF-AUC. There was a good correlation between T_{max} or MRT and ΔCFF_{max} or ΔCFF-AUC (Fig. 4) and between C_{max} and ΔCFF-AUC (Table II). On the other hand, Ka, Ke, CL_{tot}/F, and AUC were not related to ΔCFF_{max} or ΔCFF-AUC.

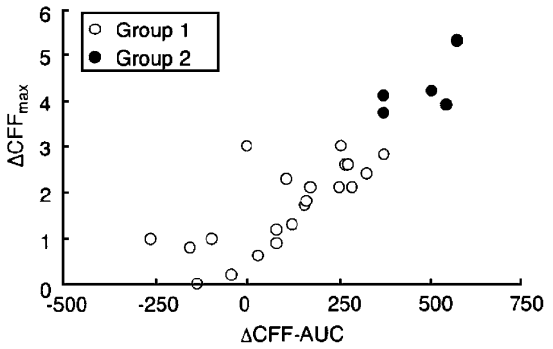

Fig. 2. Relationship between ΔCFF-AUC and ΔCFF_{max} after oral administration of loperamide HCl (2 mg) in 26 subjects. Subjects in group 1 felt no symptoms after loperamide dosing, and those in group 2 complained of drowsiness.

DISCUSSION

Schinkel *et al.* (6) studied the disposition of loperamide after oral administration in normal and *mdr1a* knockout mice. The plasma concentration of loperamide in *mdr1a* knockout mice was 2-fold higher than that in normal mice. On the other hand, the concentration in the brain was 13.5-fold higher. Thus, it is thought that the absorption of loperamide increases when P-gp function is decreased and that the distribution of loperamide in the brain will markedly increase in humans. Loperamide is an opioid agonist and affects CNS functions such as respiratory suppression when present at high levels in the brain. Sadeque *et al.* (7) have already demonstrated the drug interaction between loperamide and quinidine using ventilatory response to carbon dioxide in healthy humans. In that report, the plasma concentration of loperamide was significantly increased and the ventilatory response was decreased by the coadministration of quinidine. However, their evaluation method requires special equipment, and this test cannot be applied to patients. On the other hand, the CFF measurement system used in our study has already been confirmed to evaluate effectively the sedative effect induced by several drugs (8–11). In our method, an ordinary subject can measure the CFF value, because the measurement of CFF using the JM101 apparatus is very easy, and is able to evaluate the function of P-gp in the patients.

In the loperamide study, the CFF value of most subjects was decreased and was lowest at 1.5 to 2 h after the administration of loperamide (Fig. 1). Thereafter, the CFF value returned to the initial value. We did not determine the plasma concentration of loperamide because it was lower than the

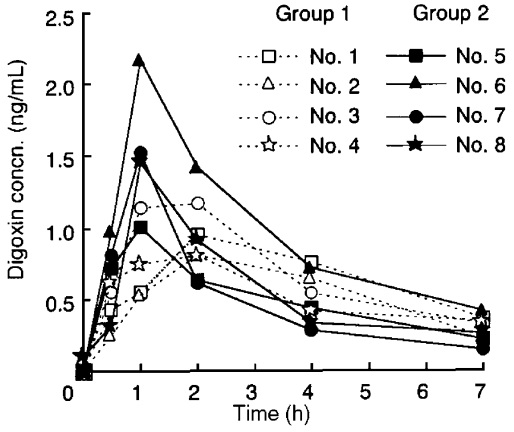

Fig. 3. Plasma concentration-time profiles of digoxin (0.25 mg) after oral administration. One tablet of digoxin containing 0.25 mg was administered to a fasted subject with 200 ml of water.

Table I. Pharmacokinetical Parameters of Digoxin in 8 Healthy Subjects after Single Oral Administration (0.25 mg)

Parameters	Group 1	Group 2
ΔCFF_{max}	2.13 ± 0.37	4.25 ± 0.72*
ΔCFF-AUC	242 ± 59	467 ± 107*
Ka (1/h)	0.89 ± 0.75	2.00 ± 0.86
Ke (1/h)	0.39 ± 0.13	0.56 ± 0.32
CL_{tot}/F (L/h)	41.8 ± 4.9	52.9 ± 16.5
C_{maxobs} (ng/ml)	0.94 ± 0.17	1.54 ± 0.48*
C_{maxsim} (ng/ml)	0.89 ± 0.18	1.30 ± 0.37
T_{maxobs} (h)	2 ± 0	1 ± 0*
T_{maxsim} (h)	1.89 ± 0.52	1.12 ± 0.11*
$AUC_{0\text{-}4h}$ (ng · h/ml)	2.78 ± 0.47	3.29 ± 1.13
$AUC_{0\text{-}7h}$ (ng · h/ml)	4.13 ± 0.56	4.35 ± 1.56
MRT (h)	3.07 ± 0.21	2.46 ± 0.65*

Each value represents the mean ± SD of four subjects.
* Significantly different from group 1, $p < 0.05$.

Table II. Correlation Coefficients Between Pharmacokinetical Parameters of Digoxin and ΔCFF-AUC or ΔCFF_{max}

Pharmacokinetical parameters	Correlation coefficient *vs.* ΔCFF-AUC	Correlation coefficient *vs.* ΔCFF_{max}
Ka (1/h)	0.300	0.512
Ke (1/h)	0.555	0.605
CL_{tot}/F (L/h)	0.357	0.660
C_{maxobs} (ng/ml)	0.810*	0.630
C_{maxsim} (ng/ml)	0.719*	0.541
T_{maxobs} (h)	–0.831*	–0.907**
T_{maxsim} (h)	–0.770*	–0.843**
$AUC_{0\text{-}4h}$ (ng · h/ml)	0.488	0.182
$AUC_{0\text{-}7h}$ (ng · h/ml)	0.252	–0.075
MRT (h)	–0.787*	–0.901**

* Significantly correlated, $p < 0.05$, **$p < 0.01$.

limit of quantification using the HPLC with UV detector. However, the CFF-time profile is similar to the ventilatory response curve reported by Sadeque *et al.* (7). Thus, the effect of loperamide on the CNS is expected to be greatest at about 2 h after oral administration. Five subjects in the loperamide study felt moderate or strong drowsiness at around 2 h post-administration, and their ΔCFF-AUC and ΔCFF_{max} values were larger than those of other subjects (Fig. 2). These results suggest that the function of the CFF value numerically expresses the sedative effect induced by loperamide. In the digoxin study, the plasma concentration of digoxin after oral administration in group 1 (nonsedative group) and group 2 (sedative group) was determined. T_{max} and MRT values of group 2 subjects were significantly lower than those in group 1, and C_{maxobs} in group 2 was significantly higher than that in group 1. Accordingly, the correlation coefficient between each pharmacokinetic parameter and ΔCFF-AUC or ΔCFF_{max} was calculated. Table II and Fig. 4 show there were negative correlations between these CFF parameters and MRT or T_{max}. MRT is a parameter that shows the residence time of a drug in a subject, thus a decrease in P-gp activity in the intestine will lead to a reduction in the residence time of digoxin in gastrointestinal tract. Moreover, there was a positive correlation between ΔCFF-AUC and C_{max}. These results strongly suggest that the activity of P-gp will be lowered in the subject whose CFF value decreases drastically after loperamide administration, and the absorption of digoxin will be more rapid in these subjects.

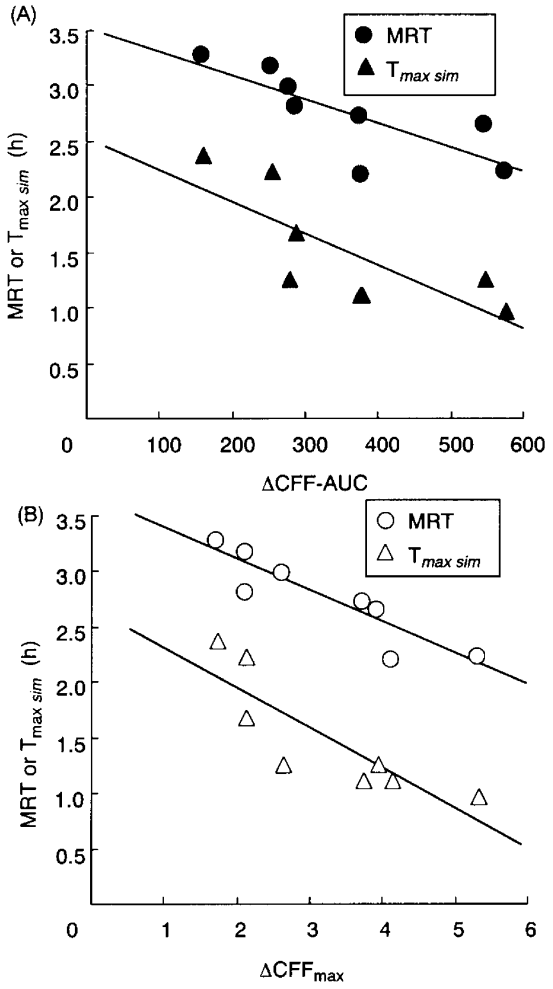

Fig. 4. Relationship between (A) ΔCFF-AUC or (B) ΔCFF_{max} of loperamide and MRT or T_{maxsim} of digoxin in eight healthy subjects.

It is known that loperamide is metabolized by CYP3A (25). Therefore, the blood concentration of loperamide will increase in subjects whose liver is dysfunctional. Recently, Tayrouz *et al.* (26) demonstrated that the oral administration of ritonavir, an HIV protease inhibitor and a typical CYP3A inhibitor, affected the plasma concentration of loperamide in healthy humans. In their report, ritonavir caused increases in the C_{max} and AUC of loperamide in comparison to a placebo. However, there were no effects of loperamide on the CNS although ritonavir is also a P-gp inhibitor. They concluded that ritonavir is not able to enhance bioavailability by the inhibition of intestinal P-gp and predominantly exerts its effect on loperamide pharmacokinetics through the inhibition of CYP3A. On the contrary, quinidine, a good inhibitor of P-gp that only inhibits CYP3A *in vitro* at much higher concentrations, enhanced the effects of loperamide on the CNS *in vivo* (7). Therefore, it is likely that if the plasma concentration of loperamide was changed among the subjects due to individual differences in CYP3A activity, there would be little variation in the effect on the CNS.

There have been a number of reports on the relationship

between gene polymorphism and the pharmacokinetics of digoxin or other P-gp substrates (15). Although they agree that the mutant C3435T at exon 26 of the P-gp gene is associated with the differences in digoxin pharmacokinetics in humans, their results are conflicting. We have started a preliminary study to examine P-gp gene polymorphism in groups 1 and 2. Currently, the genotype of P-gp gene at position 3435 of two subjects in group 1 was CC, whereas that of 3 subjects in group 2 was CT or TT (data not shown). Moreover, the mutant G2677T/A at exon 21 was also observed, but the effects on the disposition of digoxin and tacrolimus were controversial (15). Recently, it was reported that the combination of polymorphisms (haplotype) of the P-gp gene is more important for digoxin pharmacokinetics than the simple mutation of C3435T (17). Accordingly, there have been a few reports claiming that C3435T polymorphism is not associated with the disposition of digoxin (27), fexofenadine (28) and loperamide (29). Furthermore, it was reported that the P-gp gene polymorphisms at positions 2776 and 3435 had no effect on the transport activities of human P-gp expressed in LLC-PK_1 cells *in vitro*, and other genetic or environmental factors might control the expression and activity of P-gp (30). Likely as not, the P-gp polymorphism(s) that decides the interpatient variations in digoxin pharmacokinetics may be revealed in the near future. However, the interpatient variations in digoxin pharmacokinetics can not be explained solely by gene polymorphism, as P-gp expression is induced by xenobiotics such as rifampicin (31) and St. John's wort (32). Moreover, endogenous compounds also regulate the expression of P-gp. The potency of the P-gp is dependent on transport activity and expression level, which is the most important clinical factor. The analysis of gene polymorphism sometimes causes "false negative error"; for example, the blood concentration of a certain drug is very high regardless of any gene polymorphism in the metabolic enzyme. Our novel method can evaluate P-gp activity, and will be able to reduce the incidence of false negative error. Recently, it has been reported that the neurotoxicity induced by tacrolimus is related to the polymorphism of the P-gp gene in patients who received liver transplants (33). Tacrolimus is also a substrate of P-gp and neurotoxicity will occur as a result of reduced P-gp function or expression. Therefore, it seems that overdoses or unexpected adverse reactions in the CNS can be avoided by estimation of the disposition of other drugs that are substrates of P-gp using this method.

CONCLUSIONS

The sedative effect induced by the oral administration of 2 mg loperamide HCl was able to be expressed numerically, in healthy Japanese subjects, using CFF value measurement with a portable JM101 instrument. Further, the parameters such as ΔCFF_{max} and ΔCFF-AUC were found to be correlated to C_{max}, T_{max}, and MRT of digoxin. These results indicate that the absorption of digoxin will occur rapidly in subjects whose CFF values decrease markedly after the oral administration of loperamide. Therefore, this CFF measurement method is a simple tool for evaluating the function of P-gp in the intestine and brain.

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