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RESEARCH PAPER

Prediction of Nonlinear Intestinal Absorption of CYP3A4 and P-Glycoprotein Substrates from their *In Vitro* Km Values

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ABSTRACT

Purpose CYP3A4 and P-glycoprotein (P-gp) are present in the human intestine and mediate intestinal first-pass metabolism and the efflux of oral drugs, respectively. We aimed to predict whether intestinal CYP3A4/P-gp is saturated in a therapeutic dose range.

Methods Information on the Michaelis–Menten constant (Km), product of the fraction absorbed (Fa) and intestinal availability (Fg) (FaFg) of CYP3A4/P-gp substrates, and clinical AUC data including two or more different dosages for each CYP3A4/P-gp substrate was collected. The relationship between dose-normalized AUC and dose/Km value, termed the linearity index (LIN), was analyzed.

Results Among 38 CYP3A4 and/or P-gp substrates, 16 substrates exhibited nonlinear pharmacokinetics and 22 substrates exhibited linear pharmacokinetics. Substrates with a small LIN tended to exhibit linear pharmacokinetics. The smallest LIN values of a substrate that exhibited nonlinear pharmacokinetics were 2.8 and 0.77 L for CYP3A4 and P-gp substrates, respectively. A decision tree for predicting nonlinear pharmacokinetics of CYP3A4/P-gp substrates based on LIN and FaFg of drugs was proposed. This decision tree correctly predicted linearity or nonlinearity for 24 of 29 drugs.

Conclusions LIN is useful for predicting CYP3A4/P-gp-mediated nonlinearity in intestinal absorption process in humans.

KEY WORDS CYP3A4 · human · intestine · nonlinear absorption · P-gp

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INTRODUCTION

Cytochrome P450 3A4 (CYP3A4) plays critical roles in drug metabolism in the liver and in the intestine, where it accounts for about 80% of total cytochromes (1). It has been suggested that CYP3A4 substrate drugs with high hepatic intrinsic clearance have a low intestinal availability (Fg) because of intestinal metabolism by CYP3A4 (2–4). An efflux transporter, P-glycoprotein (P-gp), is expressed in the intestine and acts as a barrier against drug absorption (5). CYP3A4 and P-gp show overlapping substrate selectivity (6), and P-gp and CYP3A4 act coordinately to reduce the product of the fraction absorbed (Fa) and the Fg (FaFg) of a CYP3A4/P-gp dual substrate (7,8). Some reports have predicted the Fg value based on hepatic intrinsic clearance ($CL_{int,h}$) (3), *in vitro* metabolic studies (4), or the "Qgut" model (9–11). However, there is no standardized method for quantitatively predicting the Fa for a drug that is dependent on efflux transport by P-gp, and the prediction of the Fa is difficult.

When the dose is increased, CYP3A4/P-gp substrates with a low FaFg value may exhibit a higher FaFg value because of the saturation of intestinal CYP3A4 and/or P-gp. The same is true for drug–drug interactions (DDIs) when an inhibitor is coadministered. CYP3A4/P-gp substrates with a low FaFg value tend to show greater increases in the area under the curve (AUC) because of the inhibition of intestinal CYP3A4 and/or P-gp (12). An important question when predicting the inhibition of intestinal CYP3A4/P-gp is how to estimate the concentration of an inhibitor in the intestine, which cannot be measured directly. Even when the dose is low enough not to cause systemic inhibition of CYP3A4/P-gp, DDI can still occur because intestinal CYP3A4 and P-gp are exposed directly to a high concentration of the inhibitor after oral administration. Therefore, using the conventional method

for predicting a DDI from an inhibitor's concentration in plasma may underestimate the extent of intestinal DDIs. We previously reported a method for predicting the risk of DDI involving the inhibition of intestinal CYP3A4 and P-gp from an index termed the drug interaction number (DIN) (13). The DIN is calculated according to the following equation using the inhibition constant (Ki).

$$\mathrm{DIN} = \frac{\mathrm{dose}}{\mathrm{Ki}}$$

The DIN value can be suitable for predicting intestinal DDIs under the assumption that the inhibitor concentration in the intestine can be approximated by dividing the inhibitor dose by the intestinal volume, which is independent of inhibitors. Analysis of clinical DDI data using the DIN value derived the following empirical rules: (i) CYP3A4 inhibitors with a DIN <2.8 L have a low risk of interacting with substrates that exhibit intestinal first-pass metabolism and those with a DIN >9.4 L have a high risk, and (ii) P-gp inhibitors with a DIN <10.8 L have a low risk of interacting with P-gp substrates and those with a DIN >27.9 L have a high risk (13).

Because the DIN (dose/Ki) is useful for predicting intestinal DDIs, we reasoned that the dose/Km value would be useful for predicting nonlinear pharmacokinetics caused by the saturation of intestinal CYP3A4 and/or P-gp. We termed the dose/Km the linearity index (LIN). The use of microdose clinical studies in the development of new drugs has attracted attention recently (14–17), and microdose clinical studies have been performed to select candidates for clinical development (18–20). To maximize the value of microdose clinical studies, the ability to predict the nonlinear pharmacokinetics between the microdose and therapeutic dose is important. Without a prediction of nonlinear intestinal absorption, there is a risk that a promising candidate will be dropped from further development because of low bioavailability (F) in a microdose clinical study, despite having a higher F value in a therapeutic dose study, because of the saturation of intestinal CYP3A4 and/or P-gp. The prediction of nonlinear pharmacokinetics is also important because careful dose adjustment is needed if F increases as the dose increases in a therapeutic dose range. In this study, we analyzed the relationships between the pharmacokinetic linearity and LIN of CYP3A4/P-gp substrates. Our aim was to establish a method for predicting nonlinear intestinal absorption.

MATERIALS AND METHODS

Collection of Pharmacokinetic Data

CYP3A4 substrates written in Goodman & Gilman's *The Pharmacological Basis of Therapeutics* (11th edition) were selected for this investigation. The Km values of selected CYP3A4 substrates determined from *in vitro* metabolic studies were collected from the literature. If the Km value was not available, the Ki or IC50 value was collected and used instead. If none of these values were available, the substrate was excluded from this analysis. After checking whether these selected CYP3A4 substrates are dual CYP3A4/P-gp substrates, the Km values for P-gp were also collected as for dual substrates. If the Km value for P-gp was not available, the Ki or IC50 value for P-gp was collected and used instead of the Km value.

P-gp substrates listed in the transporter database *TP-Search* (http://125.206.112.67/tp-search/login.php) (21) were selected for this investigation. The Km values of selected P-gp substrates determined from *in vitro* studies (cell accumulation studies, transport studies, binding studies, or ATP hydrolysis studies) were collected from the literature. If the Km value for P-gp was not available, the Ki or IC50 value was collected and used instead. If none of these values were available, the substrate was excluded from this analysis. After checking whether these selected P-gp substrates are dual CYP3A4/P-gp substrates, Km values for CYP3A4 were also collected as for dual substrates. If the Km value for CYP3A4 was not available, the Ki or IC50 value for CYP3A4 was collected and used instead of the Km value.

Clinical pharmacokinetic data of CYP3A4 and/or P-gp substrates were collected from reports in the literature that provided AUC or the maximum concentration (Cmax) data that could be compared with those at different dose levels. Clinical pharmacokinetic data needed for calculating the FaFg values were also collected.

Calculation of FaFg

The F, hepatic availability (Fh), and FaFg of CYP3A4 and P-gp substrates were calculated using the following equations:

$$\mathrm{F} = \frac{\mathrm{AUCp,po} \cdot \mathrm{Dose,iv}}{\mathrm{AUCp,iv} \cdot \mathrm{Dose,po}}, \quad (1)$$

$$\mathrm{CLtot} = \frac{\mathrm{Dose,iv}}{\mathrm{AUCp,iv} \cdot \mathrm{Rb}}, \quad (2)$$

$$\mathrm{CLr} = \mathrm{CLtot} \cdot \mathrm{fe}, \quad (3)$$

$$\mathrm{CLh} = \mathrm{CLtot} - \mathrm{CLr}, \quad (4)$$

$$Fh = 1 - CLh/Qh, \quad (5)$$

$$FaFg = F/Fh, \quad (6)$$

where CLtot is the total clearance, AUCp is the AUC of plasma concentration, Rb is the blood-to-plasma drug concentration ratio, CLr is the renal clearance, fe is the urinary excretion ratio, and Qh is the hepatic blood flow rate. The notations iv and po indicate intravenous administration and oral administration, respectively. The value of 25.5 mL/min/kg was used as the hepatic blood flow rate (2). For cases in which the value of Rb was unknown, Rb was assumed to be 1 based on a report that the average of Rb values in 96 compounds in humans was near to 1 (22).

Calculation of LIN, AUC/Dose Ratio, and Cmax/Dose Ratio

It was assumed that the intestinal concentration of a substrate (C_G) can be approximated by the substrate dose (dose) divided by intestinal volume (V_G), which is independent of the inhibitor. Under this assumption, dose/Km ($=V_G \times C_G/Km$) was considered to be appropriate as the index for predicting the saturation of intestinal CYP3A4 and P-gp. This index, dose/Km, was termed LIN. If more than one Km value was collected, the geometric mean of these Km values was calculated and used to determine LIN. If more than one metabolic pathway by CYP3A4 was reported with Km values for each, the smallest Km value among the pathways contributing >20% of total clearance was used. If a Km value was not available and more than one Ki or IC50 value was collected, the geometric mean of these Ki or IC50 values was calculated and used to determine LIN. If the dose (mg) was for the salt form, the molecular weight of the salt was used to calculate LIN. The AUC/dose ratio was calculated as the ratio of AUC/dose to that at the minimum dose in each report. The Cmax/dose ratio was calculated in the same way. A substrate showing AUC/dose ratio >1.25 was judged as having nonlinear pharmacokinetics. If only a Cmax/dose ratio was available, a substrate showing a Cmax/dose ratio >1.25 was judged as having nonlinear pharmacokinetics.

Decision Tree for Predicting Nonlinear Pharmacokinetics of CYP3A4 and/or P-gp Substrates

A decision tree for predicting the nonlinear pharmacokinetics of CYP3A4 and/or P-gp substrates was developed using the LIN and FaFg values (Fig. 1). The LIN criteria for CYP3A4 and P-gp were set to the minimum values at which CYP3A4 and P-gp substrates showed nonlinear pharmacokinetics. The criteria for FaFg was set to 0.8 because FaFg must be <0.8 to produce an AUC/dose ratio >1.25 when FaFg changes to 1. The prediction accuracy of this decision tree was confirmed for CYP3A4/P-gp substrates. Among the CYP3A4 and P-gp dual substrates, only substrates whose Km (or Ki) values for both CYP3A4 and P-gp were available were included in the analysis. If we could not judge whether a drug is a dual substrate or a specific substrate, the drug was excluded from the analysis. Substrates with unavailable FaFg values were excluded from the analysis. However, buspirone, whose FaFg value was not available, was included in the analysis by assuming FaFg <0.8 because buspirone shows a large increase in the AUC when coadministered with grapefruit juice, which is often used as a specific inhibitor of intestinal CYP3A4 (23).

RESULTS

Pharmacokinetic Parameters of CYP3A4/P-gp Substrates

The values for Km (or Ki or IC50), F, Fh, and FaFg, and the oral dose used in the study to determine the F values of CYP3A4 and/or P-gp substrates are shown in Table I. The largest Km value for CYP3A4 was 188 μM (diazepam) and the smallest was 0.068 μM (ritonavir). The largest Km value for P-gp was 4100 μM (levofloxacin) and the smallest was 0.100 μM (ivermectin). The smallest FaFg value was 0.092 (oxybutynin). Eighteen of the 43 substrates whose FaFg values were calculated had an FaFg value <0.5. The pharmacokinetic linearity of the substrates used in this study is summarized in Table II.

Relationship Between Pharmacokinetic Linearity and LIN of CYP3A4 Substrates

The relationships between the LIN for CYP3A4 (LIN_{3A4}) and the AUC/dose ratio (or Cmax/dose ratio if AUC data were not available) of the CYP3A4 substrates are shown in Fig. 2. Although sildenafil and levo-acetyl α-methadol (LAAM) are dual CYP3A4/P-gp substrates, the Km (or Ki or IC50) values for P-gp were not available. However, these compounds are included in Fig. 2 because the values for CYP3A4 were available. Among the CYP3A4 substrates, the smallest LIN_{3A4} to exhibit nonlinear pharmacokinetics (AUC/dose ratio >1.25) was 2.8 L (felodipine). This suggested that CYP3A4 substrates with a LIN_{3A4} >2.8 L may show nonlinear pharmacokinetics.

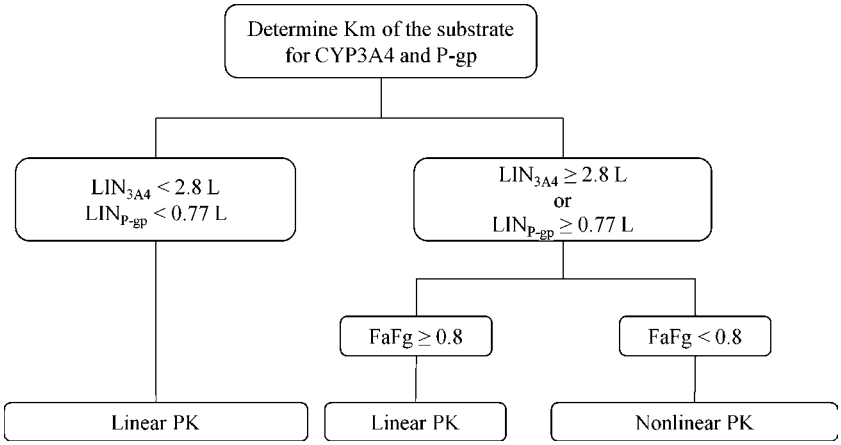

Fig. 1 Decision trees for predicting nonlinear pharmacokinetics of CYP3A4/P-gp substrates.

Relationship Between Pharmacokinetic Linearity and LIN of P-gp Substrates

The relationships between the LIN for P-gp ($LIN_{P\text{-}gp}$) and the AUC/dose ratios (or Cmax/dose ratios if AUC data were not available) of P-gp substrates are shown in Fig. 3. Although colchicine, nitrendipine, and risperidone are dual CYP3A4/P-gp substrates, the Km (or Ki or IC50) values for CYP3A4 were not available. However, these compounds are included in Fig. 3 because the values for P-gp were available. Prazosin in Fig. 3 could not be judged as CYP3A4 substrates. The smallest $LIN_{P\text{-}gp}$ that exhibited nonlinear pharmacokinetics (AUC/dose ratio >1.25) was 0.77 L (celiprolol) among the P-gp substrates. This suggested that P-gp substrates with a $LIN_{P\text{-}gp}$ >0.77 L may show nonlinear pharmacokinetics.

Relationship Between Pharmacokinetic Linearity and LIN of Dual CYP3A4/P-gp Substrates

To confirm the ability of the LIN criteria (2.8 L for CYP3A4 substrates and 0.77 L for P-gp substrates) to predict pharmacokinetic linearity, the LIN criteria were applied to dual CYP3A4/P-gp substrates. The relationships between the LIN_{3A4}, $LIN_{P\text{-}gp}$, and AUC/dose ratio (or Cmax/dose ratio if AUC data were not available) are shown in Fig. 4. All dual CYP3A4/P-gp substrates exhibiting nonlinear pharmacokinetics except for losartan had a LIN_{3A4} >2.8 L or $LIN_{P\text{-}gp}$ >0.77 L. This suggests that the LIN criteria derived from CYP3A4 substrates and P-gp substrates can be applied to dual CYP3A4/P-gp substrates.

Decision Tree for Predicting Nonlinear Pharmacokinetics of CYP3A4 and/or P-gp Substrates

In a therapeutic dose range, CYP3A4 and/or P-gp substrates with high FaFg values may exhibit linear pharmacokinetics despite the saturation of intestinal CYP3A4 and/or P-gp. Therefore, a decision tree for predicting the nonlinear pharmacokinetics of CYP3A4 and/or P-gp substrates was developed with the LIN and FaFg values included (Fig. 1). In this decision tree, substrates with a LIN_{3A4} <2.8 L and $LIN_{P\text{-}gp}$ <0.77 L are predicted to show linear pharmacokinetics. Even substrates with a LIN_{3A4} ≥2.8 L or $LIN_{P\text{-}gp}$ ≥0.77 L are predicted to show linear pharmacokinetics if the FaFg is ≥0.8. Substrates with an FaFg <0.8 are predicted to show nonlinear pharmacokinetics if the LIN values meet the criteria shown above (LIN_{3A4} ≥2.8 L or $LIN_{P\text{-}gp}$ ≥0.77). This decision tree provided true predictions for 24 of the 29 substrates tested (Table III).

DISCUSSION

In our previous study, empirical rules to predict intestinal DDI risk were derived by analyzing the clinical DDI data of CYP3A4 and/or P-gp substrates, which had low FaFg values, using the DIN (dose/Ki) values for the inhibitors (13). In this study, a LIN (dose/Km) value was applied to predict nonlinear pharmacokinetics caused by the saturation of CYP3A4 and/or P-gp based on a concept similar to DIN. This method is based on empirical rules obtained from the relationships between the LINs and the AUC/dose ratios, but it cannot distinguish the saturation of intestinal CYP3A4/P-gp from that of hepatic CYP3A4/P-gp or renal P-gp. However, intestinal CYP3A4/P-gp is considered to be saturated at a lower dose than that needed to saturate systemic CYP3A4/P-gp because intestinal CYP3A4/P-gp is exposed to a high concentration of substrates after oral administration. A hypothetical inhibitor with the highest concentration in the liver inlet must have the following properties (13): a rapid absorption rate limited by the gastric emptying rate ($ka = 0.1\ min^{-1}$), complete bioavailability ($F = 1$), no elimination ($ke =$

Table I Km (or Ki or IC50) Values and FaFg of CYP3A4 and P-gp Substrates

	Km or Ki[e] (μmol/L) CYP3A4	Method	n^f	Ref[h]	Km or IC50[e] (μmol/L) P-gp	Method	n^f	Ref[h]	F	Fh	FaFg	Dose[g] (mg)	Ref[h]
Alfentanil	22.8	Km	1	a-1	N			a-2	0.430	0.849	0.507		a-3
Alprazolam	81	Km	1	a-4	N			a-5	0.880	0.976	0.901		a-3
Atorvastatin	30.5	Km	1	a-6	288	IC50[b]	2	a-7	0.120	0.866	0.139		a-8
Buspirone	8.8	Km	1	a-9	N			a-10	0.039				a-11
Celiprolol	N			a-12	313	IC50[b]	1	a-13	0.344	0.871	0.395	100	a-8
Chlorpromazine	N			a-14	27.4	Km[d]	1	a-15	0.081	0.307	0.263	25	a-16
Cisapride	3.2	Km	1	a-17	N			a-18					
Clarithromycin	48.7	Km	1	a-19	21.9	IC50[a]	7	a-20	0.550	0.817	0.673		a-3
Colchicine	Y			a-21	7.74	Km[a,d]	2	a-22, 23	0.440	0.929	0.473		a-24
Cyclosporine	1.42	Ki	1	a-25	1.76	Km[b,d]	3	a-26-28	0.227	0.803	0.282		a-8
Delavirdine	6.8	Km	1	a-29	N			a-30					
Diazepam	188	Km	1	a-31	72.4	Km[d]	1	a-28	1.000	0.985	1.015		a-3
Digoxin	N			a-32	25.9	Km[d]	1	a-28	0.700	0.977	0.717		a-8
Diltiazem	23	Km	1	a-33	77.7	IC50[b]	1	a-34	0.380	0.852	0.446		a-3
Dipyridamole	5	Km	1	a-35	26.4	IC50[a]	3	a-20	0.430	0.923	0.466		a-36
Erythromycin	88	Km	1	a-37	37.8	IC50[a]	1	a-38	0.350	0.794	0.441	250	a-3
Etoposide	52.3	Km	3	a-39, 40	255	Km[b]	1	a-41	0.520	0.987	0.527		a-3
Felodipine	9.15	Km	1	a-42	N			a-43	0.160	0.569	0.281	27.5	a-3
Fexofenadine	N			a-44	>100	IC50[b]	1	a-45	0.280	0.904	0.310		a-8
Haloperidol	62	Km	1	a-46	33.0	Km[d]	1	a-15	0.600	0.697	0.861		a-3
Indinavir	0.522	Km	2	a-47, 48	44.0	IC50[b]	1	a-49	0.600	0.524	1.145	400	a-50
Ivermectin	40.7	Km	1	a-51	0.100	IC50[a]	1	a-52					
LAAM	19.4	Km	1	a-53	Y			a-54	0.480	0.789	0.609		a-55
Lansoprazole	102	Km	1	a-56	62.8	IC50[b]	1	a-57	0.850	0.829	1.025		a-3
Levofloxacin	N			a-58	4100	Km[b]	2	a-59, 60	0.990	0.981	1.010		a-3
Loperamide	4.05	Km	2	a-61, 62	13.8	Km[d]	1	a-28					
Loratadine	7	Km	1	a-63	3	Km[d]	1	a-64					
Losartan	82	Km	1	a-65	306	Km[b]	2	a-66	0.360	0.714	0.504	50	a-3
Methylprednisolone	100	Ki	1	a-67	134	Km[b]	2	a-68	0.820	0.820	1.000		a-11
Midazolam	2.40	Km	1	a-69	N			a-43	0.300	0.814	0.369	2	a-70
Nelfinavir	0.3	Ki	1	a-71	2.18	IC50[a]	2	a-49, 52					
Nicardipine	1.60	Ki	1	a-69	6.67	IC50[a]	9	a-20, 34, 52, 72	0.156	0.677	0.231	10	a-73
Nifedipine	10	Km	1	a-74	231	IC50[a,b]	2	a-34, 75	0.500	0.714	0.701	10	a-3
Nitrendipine	Y			a-76	68.2	IC50[b]	1	a-34	0.226	0.267	0.848		a-77
Olanzapine	N			a-78	8.30	Km[d]	1	a-15					
Oxybutynin	18.5	Km	1	a-79	N			a-80	0.063	0.682	0.092		a-11
Pafenolol	N			a-81	5.50	IC50[c]	1	a-82	0.270	0.927	0.291	25	a-83
Pimozide	0.37	Km	1	a-84	2.9	IC50[a]	1	a-52, 85					
Prazosin					20.0	Km[d]	1	a-86	0.680	0.823	0.826		a-3
Quetiapine	18	Km	1	a-87	12.3	Km[d]	1	a-15	0.090	0.255	0.353		a-11
Quinidine	78.8	Km	2	a-88, 89	9.93	Km[d]	2	a-28, 90	0.750	0.867	0.865		a-3
Quinine	83	Km	1	a-91	97.2	IC50[a]	7	a-20	0.760	0.937	0.811		a-3
Reserpine					3.07	IC50[a,b,d]	11	a-20, 38, 92, 93					
Risperidone	Y			a-94	12.4	Km[d]	1	a-15	0.660	0.795	0.830		a-3
Ritonavir	0.068	Km	1	a-48	8.95	IC50[a,b]	3	a-49, 52, 95	0.700	0.955	0.733	600	a-3
Saquinavir	1.01	Km	1	a-96	8.83	IC50[a,b]	2	a-49, 52	0.040	0.277	0.144	600	a-8
Sildenafil	14.4	Km	1	a-97	Y			a-5	0.398	0.714	0.558	50	a-98, 99

Table I (continued)

	Km or Ki[e] (μmol/L) CYP3A4	Method	n[f]	Ref[h]	Km or IC50[e] (μmol/L) P-gp	Method	n[f]	Ref[h]	F	Fh	FaFg	Dose[g] (mg)	Ref[h]
Tacrolimus	1.5	Km	1	a-100	0.74	IC50[b]	1	a-101	0.131	0.966	0.136		a-8
Talinolol	N			a-102	72	IC50[c]	1	a-82	0.453	0.929	0.488	100	a-8
Toremifene	124	Km	1	a-103	7.5	Km[d]	1	a-104					
Trazodone	163	Km	1	a-105	N			a-10	0.770	0.945	0.815		a-3
Triazolam	175	Km	1	a-69	N			a-5	0.475	0.866	0.549		a-8
Verapamil	44.7	Km	1	a-106	2.85	Km[d]	2	a-28, 107	0.210	0.353	0.595		a-3
Zolpidem	140	Km	1	a-108	N			a-10	0.720	0.813	0.886		a-3

[a] cell accumulation

[b] transport study

[c] binding study

[d] ATP hydrolysis

[e] Y, substrate; N, nonsubstrate or poor substrate

[f] number of reported values used for calculating geometric mean

[g] oral dose used in the study determining F values of nonlinear substrates

[h] References are shown in the Appendix

0 min^{-1}), no protein binding (fu = 1), and a small distribution volume close to the extracellular fluid volume (Vd = 14 L). Therefore, the hypothetical inhibitor's maximum unbound concentration in the liver inlet ($[I]_{in,u,max}$) can be calculated as fu × dose × ka/Qh, and the smallest DIN value needed to increase the AUC of a substrate to ≥125% can be calculated as 4.5 L, considering that $[I]_{in,u,max}/Ki$ must be >0.25 (13). Because the renal artery concentration is considered to be lower than the hepatic inlet concentration, which includes drugs coming from portal blood flow and the hepatic artery, renal DDIs will occur only when DIN >4.5 L. Therefore, in the present study, the nonlinear pharmacokinetics for drugs having LIN values <4.5 L could be ascribed to the saturation of intestinal CYP3A4/P-gp.

The solubility of an inhibitor is important to intestinal DDIs because it can limit the intestinal concentration of the inhibitor. In our previous study, some inhibitors did not cause DDIs regardless of their high DIN value, probably because of their low solubility. This is also true for the saturation of intestinal CYP3A4 and/or P-gp. For drugs showing solubility-limited absorption, the dose-proportional concentration in the intestine cannot be achieved, and AUC/dose decreases with an increased dose. In this study, few substrates exhibited a decreased AUC/dose ratio of <0.8 with an increase in dose, and most of these substrates were considered to have good solubility. When this prediction method is applied to new drug development, the solubility of the drug must be taken into account. This analysis cannot distinguish the following two cases: (i) the intestinal concentration close to dose/V_G cannot be achieved because of low solubility, and (ii) the uptake transporter in the intestine might be saturated.

In our previous study, CYP3A4/P-gp substrates with relatively low FaFg values were selected, and the DDI data of these substrates were collected because the effect of intestinal DDIs was considered to be large for these substrates (13). In the present study, the FaFg values were not taken into account when selecting substrates because we considered the possibility that some substrates may show a high FaFg value because of saturation of intestinal CYP3A4 and/or P-gp. Therefore, substrates showing higher FaFg values in a therapeutic dose range despite a high LIN may exhibit lower FaFg values as the dose decreases further (e.g., microdoses).

The smallest LIN_{3A4} of a CYP3A4 substrate that showed nonlinear pharmacokinetics was 2.8 L (felodipine). Felodipine is a CYP3A4-specific substrate selected as the victim drug in our previous study, and it has a low FaFg because of intestinal metabolism by CYP3A4 (13). The LIN_{3A4} criterion (2.8 L) is similar to that for DIN (2.8 L), which divides low risk from medium risk for DDIs mediated by intestinal CYP3A4. Therefore, the risk of intestinal DDIs and nonlinear intestinal absorption can be predicted by the common criterion. The smallest $LIN_{P\text{-}gp}$ of a P-gp substrate showing nonlinear pharmacokinetics was 0.77 L (celiprolol). For celiprolol, the contribution of metabolism to the pharmacokinetics is minimal (24,25). The $LIN_{P\text{-}gp}$ criterion (0.77 L) is much smaller than the DIN criterion (10.8 L), which divides low risk from medium risk for DDIs mediated by intestinal P-gp. In this study, the Ki value of celiprolol

Table II Relationship Between LIN and AUC/dose (Cmax/dose) Ratio of CYP3A4 and P-gp Substrates

	Oral dose	LIN_{3A4}	$LIN_{P\text{-}gp}$	AUC	AUC/dose		Cmax	Cmax/dose		Ref.[a]
	mg	L	L	ng×h/mL	ng×h/mL/mg	ratio	ng/mL	ng/mL/mg	ratio	
Alprazolam	0.4	0.016					6.8	17.0	1.00	a-109
	0.8	0.032					12.7	15.9	1.07	
Atorvastatin	5	0.293	0.031	17.33	3.47	1.00	2.64	0.528	1.00	a-110
	10	0.587	0.062	34.57	3.46	1.00	3.42	0.342	0.65	
	20	1.174	0.124	50.87	2.54	0.73	11.29	0.565	1.07	
	40	2.348	0.248	117.91	2.95	0.85	27.05	0.676	1.28	
Buspirone	5	1.285		0.69	0.138	1.00				a-111
	7.5	1.928		1.0	0.133	0.97				
	15	3.856		2.3	0.153	1.11				
	20	5.142		3.4	0.170	1.23				
	30	7.713		4.7	0.157	1.14				
Celiprolol	50		0.384	47.6	0.952	1.00	13.1	0.262	1.00	a-112
	100		0.768	304	3.04	3.19	116	1.16	4.43	
	200		1.536	1830	9.15	9.61	295	1.48	5.63	
	400		3.072	6810	17.0	17.88	855	2.14	8.16	
Chlorpromazine	25		2.568	27.8	1.11	1.00	4.31	0.172	1.00	a-16
	50		5.136	81.8	1.64	1.47	11.9	0.238	1.38	
	100		10.271	247	2.47	2.22	37.9	0.379	2.20	
Colchicine	0.5		0.162	14.1	28.2	1.00	2.2	4.40	1.00	a-113
	1		0.324	26.3	26.3	0.93	3.9	3.90	0.89	
	1.5		0.485	47.3	31.5	1.12	6.7	4.47	1.02	
Delavirdine	100	26.609					1437	14.4	1.00	a-114
	150	39.913					4311	28.7	2.00	
	200	53.218					8843	44.2	3.08	
	250	66.522					11606	46.4	3.23	
	300	79.826					16027	53.4	3.72	
Digoxin	0.25		0.012	8.3	33.2	1.00				a-115
	0.5		0.025	16.41	32.8	0.99				
Erythromycin	250	3.871	9.014	5400	21.6	1.00				a-116
	500	7.742	18.027	13200	26.4	1.22				
	1000	15.483	36.055	28600	28.6	1.32				
Etoposide	25	0.812	0.167	9650	386	1.00	1060	42.4	1.00	a-117
	50	1.623	0.333	14110	282	0.73	2050	41.0	0.97	
	75	2.435	0.500	28560	381	0.99	3420	45.6	1.08	
Felodipine	2.5	0.711		7.7	3.08	1.00	2.4	0.960	1.00	a-118
	5	1.422		14.1	2.82	0.92	7.3	1.46	1.52	
	10	2.844		48.6	4.86	1.58	12.2	1.22	1.27	
Indinavir	400	1247.225	14.811	4211	10.5	1.00	2750	6.87	1.00	a-119
	700	2182.643	25.919	11012	15.7	1.49	6046	8.64	1.26	
	1000	3118.062	37.027	20563	20.6	1.95	10054	10.1	1.46	
Ivermectin	6	0.169	68.564	347	57.8	1.00	18.3	3.05	1.00	a-120
	12	0.337	137.127	513	42.8	0.74	30.6	2.55	0.84	
	15	0.421	171.409	820	54.7	0.95	48.5	3.23	1.06	
LAAM	20	2.911		393	19.7	1.00	39.0	1.95	1.00	a-55
	40	5.823		944	23.6	1.20	63.0	1.58	0.81	
Lansoprazole	15	0.398	0.647	1332.4	88.8	1.00	472	31.5	1.00	a-121
	30	0.796	1.293	3238	108	1.22	1194	39.8	1.26	

Table II (continued)

	Oral dose	LIN_{3A4}	$LIN_{P\text{-}gp}$	AUC	AUC/dose		Cmax	Cmax/dose		Ref.[a]
	mg	L	L	ng×h/mL	ng×h/mL/mg	ratio	ng/mL	ng/mL/mg	ratio	
Levofloxacin	50		0.033	4700	94.0	1.00	570	11.4	1.00	a-58
	100		0.066	7460	74.6	0.79	1220	12.2	1.07	
	200		0.132	19880	99.4	1.06	2040	10.2	0.89	
Losartan	25	0.661	0.177	201	8.04	1.00	85	3.40	1.00	a-122
	50	1.323	0.355	354	7.08	0.88	198	3.96	1.16	
	100	2.645	0.709	1069	10.7	1.33	801	8.01	2.36	
	200	5.291	1.418	2231	11.2	1.39	1395	6.98	2.05	
Methylprednisolone	1	0.027	0.020							a-123
	2	0.053	0.040				22.8	11.4	1.00	
	4	0.107	0.080				34.4	8.60	0.75	
	8	0.214	0.160				68.2	8.53	0.75	
	24	0.641	0.479				174	7.25	0.64	
	80	2.136	1.595				794	9.93	0.87	
Midazolam	7.5	9.593		92	12.3	1.00				a-124
	15	19.185		188	12.5	1.02				
	30	38.371		503	16.8	1.37				
Nelfinavir	250	1467.694	201.815	3100	12.4	1.00				a-125
	500	2935.389	403.630	16300	32.6	2.63				
	750	4403.083	605.445	40670	54.2	4.37				
	1000	5870.777	807.260	66630	66.6	5.37				
Nicardipine	10	12.113	2.905	45	4.52	1.00				a-73
	20	24.225	5.809	151	7.54	1.67				
	30	36.338	8.714	306	10.2	2.26				
	40	48.451	11.618	498	12.5	2.76				
Nifedipine	5	1.444	0.063	78	15.6	1.00	45	9.00	1.00	a-126
	10	2.887	0.125	179	17.9	1.14	123	12.3	1.37	
	20	5.775	0.250	424	21.2	1.36	252	12.6	1.40	
Nitrendipine	5		0.203	46.07	9.21	1.00	4.06	0.812	1.00	a-127
	10		0.407	65.32	6.53	0.71	7.08	0.708	0.87	
Olanzapine	2.5		0.964	146	58.4	1.00	3.86	1.54	1.00	a-128
	5		1.928	293	58.6	1.00	6.94	1.39	0.90	
	10		3.856	578	57.8	0.99	15.3	1.53	0.99	
Oxybutynin	2	0.275		8.21	4.11	1.00	5.22	2.61	1.00	a-129
	3	0.413		10.53	3.51	0.86	6.67	2.22	0.85	
	6	0.825		19.16	3.19	0.78	9.28	1.55	0.59	
	9	1.238		35.92	3.99	0.97	16.32	1.81	0.69	
Pafenolol	25		13.468	353	14.1	1.00	35.4	1.42	1.00	a-83
	50		26.936	862	17.2	1.22	116	2.32	1.63	
	100		53.872	2760	27.6	1.96	419	4.19	2.95	
Prazosin	0.5		0.065	38	76.0	1.00	6.2	12.4	1.00	a-130
	1		0.130	64	64.0	0.84	9.4	9.40	0.76	
	2		0.261	169	84.5	1.11	20.3	10.2	0.82	
	4		0.522	254	63.5	0.84	37.1	9.28	0.75	
Quinine	250	7.589	6.482	18500	74.0	1.000	1600	6.40	1.00	a-131
	500	15.177	12.963	30200	60.4	0.816	2700	5.40	0.84	
	1000	30.355	25.927	92400	92.4	1.249	4970	4.97	0.78	
Risperidone	1		0.196	24.48	24.5	1.00	4.80	4.80	1.00	a-132

Table II (continued)

	Oral dose	LIN_{3A4}	$LIN_{P\text{-}gp}$	AUC	AUC/dose		Cmax	Cmax/dose		Ref.[a]
	mg	L	L	ng×h/mL	ng×h/mL/mg	ratio	ng/mL	ng/mL/mg	ratio	
	2		0.393	45.01	22.5	0.92	8.59	4.30	0.89	
	3		0.589	59.75	19.9	0.81	13.07	4.36	0.91	
Ritonavir	200	4079.641	31.011	18700	93.5	1.00	2000	10.0	1.00	a-133
	300	6119.462	46.516	33400	111	1.19	4400	14.7	1.47	
	400	8159.282	62.021	68900	172	1.84	9000	22.5	2.25	
	500	10199.103	77.526	83700	167	1.79	9600	19.2	1.92	
Saquinavir	600	889.950	101.271	714.2	1.19	1.00				a-134
	1200	1779.900	202.542	4092	3.41	2.86				
Sildenafil	25	3.658		361	14.4	1.00				a-99
	50	7.316		738	14.8	1.02				
	100	14.632		1685	16.9	1.17				
	200	29.264		3755	18.8	1.30				
Tacrolimus	3	2.488	5.042	169	56.3	1.00	14.5	4.83	1.00	a-135
	7	5.804	11.765	355	50.7	0.90	31.2	4.46	0.92	
	10	8.292	16.807	485	48.5	0.86	45.1	4.51	0.93	
Talinolol	25		0.955	500	20.0	1.00	46	1.84	1.00	a-136
	50		1.910	1238	24.8	1.24	144	2.88	1.57	
	100		3.821	3282	32.8	1.64	323	3.23	1.76	
	400		15.284	14686	36.7	1.84	1615	4.04	2.19	
Toremifene	40	0.795	13.138	3400	85.0	1.00	233	5.83	1.00	a-137
	120	2.384	39.413	10500	87.5	1.03	855	7.13	1.22	
Trazodone	50	0.751		7120	142	1.00	940	18.8	1.00	a-138
	100	1.502		13070	131	0.92	1330	13.3	0.71	
Triazolam	0.125	0.002		5.5	44.0	1.00	1.25	10.0	1.00	a-139
	0.25	0.004		10.6	42.4	0.96	2.6	10.4	1.04	a-140
Zolpidem	10	0.187		408	40.8	1.00	125	12.5	1.00	a-141
	20	0.374		889	44.5	1.09	232	11.6	0.93	

[a] References are shown in the Appendix

was used instead of the Km value because the latter value was not available in the literature, and this may be one reason for the discrepancy between the LIN and DIN criteria for P-gp. This Ki value was determined by an *in vitro* inhibition transport study of taxol from the basolateral to apical direction in Caco-2 cells (26).

There appears to be large variability in determining the Ki (IC50) value (12). The apparent Km values of P-gp substrates were reported to increase when the P-gp expression level in the cell studies was increased (27,28). This phenomenon can be explained by a mechanism in which a higher P-gp level causes a lower drug concentration in the cell. With an appropriate kinetic model, the derived Km values of P-gp substrates based on the intracellular free concentration were about the same for all tested cells expressing various levels of P-gp (29). In addition to P-gp level, changes in the experimental conditions such as the pH of the extracellular buffer and aqueous boundary layers caused one order of magnitude variation in the apparent affinity for P-gp (Km, app). However, the Km values derived by fitting the concentration data into a compartmental model that accounted for the aqueous boundary layers, cell membranes, and cellular retention were about the same for all conditions (30). The variability in the reported Km (Ki) values may be explained by the difference in the P-gp expression level of the cells and experimental conditions. Analysis by the kinetic model may contribute to reducing such variability in determining Km. The $LIN_{P\text{-}gp}$ criterion may change if a precise Km value for celiprolol is determined. However, at this stage, this low $LIN_{P\text{-}gp}$ criterion may be useful for avoiding false-negative predictions.

Celiprolol and talinolol, which were selected as P-gp substrates in this study, are transported in the absorptive direction by the OATP family (31,32). Substrates of an absorptive transporter (e.g., OATP members) are thought to show decreased FaFg as the dose increases. By contrast,

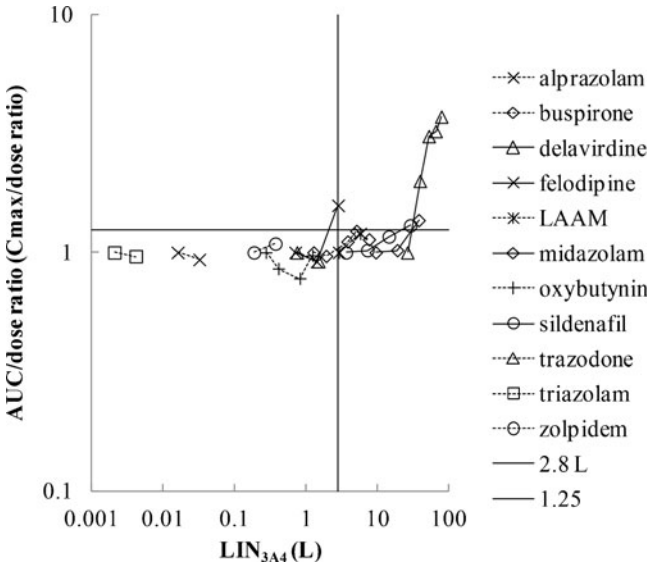

Fig. 2 Relationships between the LIN_{3A4} and the AUC/dose ratios of CYP3A4 substrates. The horizontal line represents the ratio 1.25, which divides the linear and nonlinear pharmacokinetics. The vertical line represents a LIN_{3A4} of 2.8 L, which was the smallest LIN_{3A4} resulting in nonlinear pharmacokinetics (felodipine).

an increased AUC/dose ratio of celiprolol and talinolol with increased dose was observed. These clinical data may reflect the saturation of transport by P-gp and not by OATP members. Future studies are needed to establish a method to predict the saturation of absorptive transport.

Using the decision tree presented in this study, we achieved true prediction for pharmacokinetic linearity in 24 of the 29 substrates. This method is simple and has broad utility. The LIN_{3A4} values of tacrolimus (8.3 L), buspirone (7.7 L), and LAAM (5.8 L), which produced false-positive predictions for all three substrates, were smaller than the DIN criterion (9.4 L) for CYP3A4, which divides medium and high risk (13). Therefore, these cases do not necessarily disprove the usability of the LIN method. It might be better to classify the CYP3A4 substrates whose LIN_{3A4} values are between 2.8 and 9.4 L into gray-zone compounds. Losartan produced false-negative predictions, but its LIN_{3A4} value (2.6 L) and $LIN_{P\text{-}gp}$ value (0.71 L) were not far from the criteria for CYP3A4 (2.8 L) and P-gp (0.77 L), respectively. Moreover, the departure from linearity at the dose is relatively small (AUC/dose ratio = 1.33). Except for the example of losartan, these LIN criteria seemed to be satisfactory. Indinavir has a high LIN_{3A4} value but was predicted to be linear because of a high FaFg value close to 1 (Table I). The plasma unbound Cmax of indinavir is higher than the Ki value (33), and the saturation of hepatic CYP3A4 may be the cause of the false-negative prediction for indinavir. For predicting nonlinear pharmacokinetics caused by both saturation of intestinal CYP3A4/P-gp and hepatic CYP3A4, this decision tree needs to be used in combination with the reported prediction method for nonlinear pharmacokinetics caused by saturation (inhibition) of hepatic metabolism (34,35).

These results suggest that the developed decision tree is of practical use. However, the Km and Ki values were collected from the literature and the method for Km or Ki determination differed between reports. Therefore, the LIN criteria derived in this study may not be absolute. As discussed earlier, different experimental conditions may affect the Km values of P-gp substrates. Nonspecific binding to microsomes may cause higher apparent Km values of CYP3A4 substrates in metabolic studies (36). When the decision tree is used in the drug-development process, to avoid false-negative predictions, it may be better to set laboratory-specific LIN criteria by determining the Km values of substrates that showed nonlinear pharmacokinetics in this study.

The use of microdose clinical studies in the development of new drugs has attracted attention recently (14–17). The

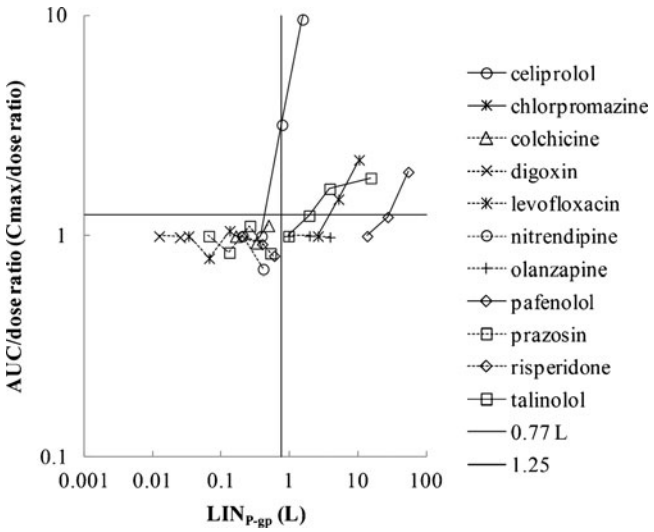

Fig. 3 Relationships between the $LIN_{P\text{-}gp}$ and the AUC/dose ratios of P-gp substrates. The horizontal line represents the ratio 1.25, which divides the linear and nonlinear pharmacokinetics. The vertical line represents a $LIN_{P\text{-}gp}$ of 0.77 L, which was the smallest $LIN_{P\text{-}gp}$ resulting in nonlinear pharmacokinetics (celiprolol).

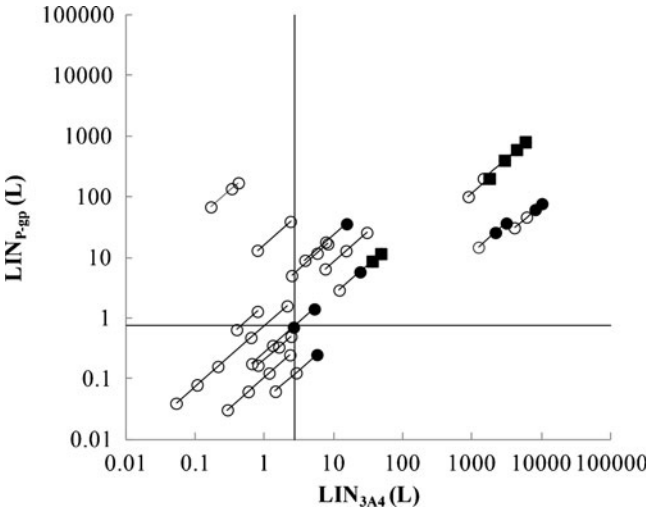

Fig. 4 Relationships between the LIN_{3A4}, $LIN_{P\text{-}gp}$, and AUC/dose ratios of CYP3A4/P-gp dual substrates. The horizontal and vertical lines represent a $LIN_{P\text{-}gp}$ of 0.77 L and a LIN_{3A4} of 2.8 L, respectively. AUC/dose ratio <1.25 (*white circle*); 1.25 ≤AUC/dose ratio <2 (*black circle*); AUC/dose ratio ≥2 (*black square*). Each diagonal line represents one substrate.

prediction method presented in this study might be useful for maximizing the value of data obtained in clinical microdose studies for predicting whether the dose-normalized AUC value (AUC/dose) obtained at the microdose level is similar to that obtained at the therapeutic dose level. Quinidine and verapamil show lower AUC/dose values at a microdose than at a therapeutic dose level (Maeda *et al.*, Clin Pharmacol Ther. Accepted for publication). Fexofenadine (37–39) shows similar AUC/dose values at the microdose and therapeutic dose levels. The decision tree presented in this study was applied successfully to these substrates to predict nonlinear pharmacokinetics (data not shown). If the risk of nonlinear pharmacokinetics is predicted, a strategy of then trying more precise and quantitative prediction methods would be preferable. Such quantitative prediction methods have not been standardized, although the analysis of the nonlinear pharmacokinetics of talinolol using GastroPlus has been reported (40,41). It is expected that a quantitative method for predicting the nonlinear absorption from the intestine will be established in the future.

CONCLUSION

In this study, a decision tree for predicting the nonlinear pharmacokinetics of CYP3A4 and/or P-gp substrates was developed using the LIN and FaFg values. According to the tree, substrates with LIN_{3A4} <2.8 L and $LIN_{P\text{-}gp}$ <0.77 L are predicted to show linear pharmacokinetics. In the case of substrates with LIN_{3A4} ≥2.8 L or $LIN_{P\text{-}gp}$ ≥0.77 L, substrates with FaFg ≥0.8 are predicted to show linear pharmacokinetics and substrates with FaFg <0.8 are predicted to show nonlinear pharmacokinetics. This simple decision tree, by which the saturation of intestinal CYP3A4 and P-gp can be predicted, will be useful in predicting the dose–AUC relationship of new drug candidates.

Table III Prediction of the Linearity of CYP3A4/P-gp Substrates Based on LIN and FaFg

	LIN_{3A4} <2.8 L $LIN_{P\text{-}gp}$ <0.77 L	LIN_{3A4} ≥2.8 L or $LIN_{P\text{-}gp}$ ≥0.77 L	
		FaFg ≥0.8	FaFg <0.8
Linear PK	True negative	True negative	False positive
	alprazolam atorvastatin digoxin etoposide levofloxacin oxybutynin trazodone triazolam zolpidem	lansoprazole methylprednisolone quinine	buspirone LAAM tacrolimus
Nonlinear PK	False negative	False negative	True positive
	losartan	indinavir	celiprolol chlorpromazine erythromycin felodipine midazolam nicardipine nifedipine pafenolol ritonavir saquinavir sildenafil talinolol

APPENDIX

Table References

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