Relationship between time after intake of grapefruit juice and the effect on pharmacokinetics and pharmacodynamics of nisoldipine in healthy subjects

A clinical study was performed in eight healthy volunteers to investigate the effect of various timing of grapefruit juice intake on nisoldipine pharmacokinetics and pharmacodynamics, and to validate our pharmacokinetic model. The subjects were given 10 mg oral nisoldipine with water (control), or 5 mg oral nisoldipine with 200 mL grapefruit juice (G0) or with water at 14 (G14), 38 (G38), 72 (G72) or 96 hours (G96) after a 7-day period of thrice-daily intake of grapefruit juice. Grapefruit juice ingestion did not affect heart rate or the effect area during the first 8 hours of heart rate after nisoldipine administration, although significant decreases of systolic and diastolic blood pressure were caused in G0 by co-administration of grapefruit juice with nisoldipine. Headaches were reported by 3, 2, and 1 persons in G0, G14, and G38, respectively, but no subjects in G72 and G96 reported headaches. Compared with the control group, the maximum plasma concentration of nisoldipine was significantly increased after grapefruit juice intake in G0 and G14, and the plasma concentration was significantly increased at each time in G0 to G72. Therefore the effect of grapefruit juice decreased time dependently and lasted for at least 3 days after intake. Furthermore, our model gave predicted values in good agreement with the observed values. It is therefore necessary to withhold grapefruit juice for at least 3 days before administration of the drug to prevent grapefruit juice-nisoldipine interaction. (Clin Pharmacol Ther 2000;67:201-14.)

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There have been a number of reports concerning the effect of the intake of grapefruit juice on the pharmacokinetic parameters (total body clearance and gastrointestinal clearance) of drugs. That is, oral intake of grapefruit juice results in increased maximum plasma concentrations ($C_{\text{max}}$) and areas under the plasma concentration–time curve (AUC) of a wide range of clinically used drugs, including dihydropyridine-type calcium antagonists such as felodipine,1-5 nifedipine,1,6 nimodipine,7 nitrendipine,8 and terfenadine,9 cyclosporine (INN, ciclosporin),10,11 diazepam,12 midazolam,13 triazolam,14 saquinavir,15 ethinyl estradiol (INN, ethinylestradiol),16 caffeine,17 tacrolimus,18 simvastatin,19 simvastatin acid,19 lovastatin,20 lovastatin acid,20 carbamazepine,21 and buspirone.22 In contrast, the ingestion of grapefruit juice does not alter the plasma concentrations of felodi-
pine,5 nifedipine,6 midazolam,13 or cyclosporine11 after intravenous drug administration. These findings suggest that grapefruit juice influences drug absorption in the intestine. In particular, drugs with low bioavailability, such as nisoldipine,23 nimodipine,7 terfenadine,9 saquinavir,15 simvastatin,19 simvastatin acid,19 lovastatin,20 and lovastatin acid20 show a 1.5- to 12-fold increase of AUC when administered after intake of grapefruit juice.24 These drugs are substrates of cytochrome P450 (CYP) 3A (CYP 3A4 and/or 3A5) and either substrates or inhibitors of P-glycoprotein.25 Therefore the mechanism of grapefruit juice–drug interaction is thought to involve inhibition of drug metabolism by CYP3A or inhibition of drug efflux by P-glycoprotein.26-28 The relative contributions of these mechanisms to the increase in the plasma concentration of drugs in the presence of grapefruit juice seem to be determined by the differences in the affinities of the drugs for the two proteins. Dihydroxybergamottin,29 bergamottin,30 GF-I-1 (4-[[6-hydroxy-7-[[1-[[1-hydroxy-1-methyl]ethyl]]-4-methyl-6-(7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl]-4-hexenyl]oxy]]-3,7-dimethyl-2-octenyl]oxy]-7H-furo[3,2-g][1]benzopyran-7-one),31 and GF-I-4 (4-[[6-hydroxy-7-[[4-methyl-1-(1-methylethenyl)-6-(7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl]-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]oxy]-7H-furo[3,2-g][1]benzopyran-7-one)31 have been identified as candidate inhibitors of CYP3A. We found that these furanocoumarin derivatives also inhibit P-glycoprotein function in Caco-2 cells (Matsuo H, et al, unpublished data, September 1999). However, calcium antagonists of the dihydropyridine type, used as antihypertensive agents, are only weak inhibitors of P-glycoprotein32-34 and may be poor substrates, so the grapefruit juice–calcium antagonist interaction is likely to be predominantly determined by CYP3A4 inhibition.

To date there has been little information on the duration of the grapefruit juice–drug interaction after intake of grapefruit juice.3 Quantitative prediction of grapefruit juice–drug interaction is important to protect patients from possible adverse effects. We developed a pharmacokinetic model based on the irreversible inhibition of intestinal CYP3A4 by grapefruit juice components.35 In this study, we assess the relationship between the times of grapefruit juice and drug intake and the effect on the pharmacokinetic and pharmacodynamic characteristics of the drug, based on our pharmacokinetic model, using nisoldipine as a model drug. The parameter values estimated from the model were used to predict the duration of the grapefruit juice effect on the drug clearance.

METHODS
Experimental design

Eight healthy volunteers (five men [age range, 22 to 26 years; mean ± SD age, 23.4 ± 2.24 years; weight range, 69.0 to 81.2 kg; mean ± SD weight, 72.7 ± 19.6 kg] and three women [age range, 22 to 24 years; mean ± SD age, 22.7 ± 0.889 years; weight range, 44.3 to 51.6 kg; mean ± SD weight, 47.9 ± 8.88 kg]) were selected for the
study. None of the subjects had a previous history of drug allergy or drug or alcohol abuse. Two men smoked cigarettes and the other subjects were nonsmokers. All subjects abstained from ingestion of nicotine and caffeine during the study. They received written and oral information about the purpose and design of the study, as well as possible adverse drug effects, before written informed consent was obtained. The study was performed at Kyushu Clinical Pharmacology Research Clinic, Chiyogu, Chuo-ku, Fukuoka-shi, Japan, after gaining the approval of the Committee of Medical Co. LTA Clinical Pharmacology Center Institutional Review Board, Chiyogu, Chuo-ku, Fukuoka, Japan.

The subjects were instructed to refrain completely from consumption of all citrus fruit, citrus products, and alcohol for at least 2 days before the first experimental day and throughout the entire study period. The subjects were also instructed to refrain completely from smoking and from ingesting anything that contained caffeine for 8 hours after drug administration. During the first 4 hours after drug intake the subjects remained in a semirecumbent position as much as possible; thereafter they were allowed to walk around freely. Each subject participated in six single-dose experiments at least 1 week apart. The experiments always started in the morning after an overnight fast. On the study days, the subjects were given 10 mg oral nisoldipine (Baymycard, Bayer, Osaka, Japan) with water (control group), or 5 mg oral nisoldipine with 200 mL grapefruit juice (Dole, Snow Brand Milk Product Co Ltd, Sapporo, Japan) (G0) or 5 mg nisoldipine was administered 14 (G14), 38 (G38), 72 (G72), or 96 hours (G96) after the last ingestion of a 7-day period of thrice-daily intake of grapefruit juice (9 AM, 1 PM, and 7 PM), as shown in Fig 1. Water was allowed ad libitum during the study days. All of the grapefruit juice used in this study was purchased at the same time in October 1998.

In G96, one volunteer (male) was not given nisoldipine at 96 hours after a 7-day period of thrice-daily intake of grapefruit juice but was given nisoldipine at 72 hours after a 7-day period of thrice-daily intake of grapefruit juice. His data was therefore excluded from the analysis of G96.

Assessments

Blood samples (4 mL) for analysis of nisoldipine and its metabolites (BAY r-9425, BAY r-9590, and BAY o-3199) were collected from an antecubital cannula before and 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours after drug intake. Samples were immediately protected from light and centrifuged at 3000 rpm for 10 minutes at 4°C and, after separation under a sodium lamp, the plasma was frozen (–20°C) until analysis. Quantitative analysis of nisoldipine, BAY r-9425, BAY r-9590, and BAY o-3199 by use of a gas chromatographic method was performed as described previously,36 with slight modification. In brief, 100 µL of 5 mol/L sodium hydroxide was added to 500 µL of blood samples and mixed well after addition of 500 µL toluene, including 50 ng/mL nimodipine (internal standard). Samples were centrifuged at 1670g for 15 minutes and then left for 1 hour at –80°C. The organic (toluene) layer (5 µL) was used for the analysis by capillary gas chromatography with electron-captured detection (GC-ECD).

The GC-ECD system consisted of an HP 5890 plus autoinjector (Hewlett-Packard Japan Ltd, Tokyo, Japan). The column was a J & W DB1 (0.1 µm, 0.25 mm x 30 m; J & W Scientific, Folson, Calif). The column temperature was programmed from 160°C to 270°C at 10°C per minute.

The limits of quantification were 0.1, 1.0, 1.0, and 1.0 ng/mL for nisoldipine, BAY r-9425, BAY r-9590, and BAY o-3199, respectively. For all measurements, coefficients of variation were less than 10%, and within-run accuracy was always within ±10%. Moreover, coefficients of variation for between-day precision measurements were less than ±10%.

Measurements of systolic and diastolic blood pressures and heart rate were made with the subjects in a semirecumbent position immediately before blood sampling. The blood pressure was measured with use of an autophsygmomanometer. The morning baseline values were measured after subjects had rested for 5 minutes in the semirecumbent position. Adverse reactions were identified both by open questioning and by spontaneous reports from the subjects.

Calculations

The maximum plasma concentration (Cmax) and the time to reach Cmax (tmax) were determined from the highest observed value in the individual plasma concentration–time profiles. When two maximal values were observed, the first was used to define tmax. The terminal half-life (t1/2) was calculated as ln2/k, in which k is the terminal rate constant determined by logarithmic regression analysis of the points after tmax, based on the one-compartment model.4

The area under the plasma concentration–time curve extrapolated to infinity [AUC(0–∞)] was calculated as follows:

\[ AUC(0–∞) = AUC_t + C_t/k \]

in which AUC_t is the area under the curve from time zero to the time of the last blood sample, and C_t is the...
plasma concentration at the corresponding time, calculated with use of the regression equation for estimation of the elimination rate constant.

Individual time profiles of effects on systolic blood pressure, diastolic blood pressure, and heart rate were calculated on the basis of changes from the morning basal values. The maximum effect of nisoldipine on blood pressure and heart rate (Emax) was determined from the highest observed value in the individual time profile, and the effect area during the first 8 hours after drug administration [AUE(0-8)] was calculated by the linear trapezoidal method.

**Statistical analysis**

All calculated pharmacokinetic parameters except tmax were assumed to follow log-normal distribution and were log-transformed before analysis. Treatments were compared with use of the Student t distribution in terms of 95% confidence intervals for the ratio of the estimated mean parameter values. Results from the control experiments (experiment 1) were compared in pairs and sequentially with results from drug administrations that included grapefruit juice (experiments 2 to 6). A difference from the control group in the kinetic parameters was accepted as significant if all comparisons up to and including that time point had a P < .05.

In the analysis of the hemodynamic parameters Emax and AUE(0-8), the values obtained in the control experiment (experiment 1) were compared in pairs and sequentially with values after grapefruit juice intake (experiments 2 to 6) with the Student t test. The level of significance was set at P < .05. No statistical analysis was made of adverse events.

**Analysis in the pharmacokinetic model**

Our previously developed model, based on irreversible inhibition of the drug-metabolizing enzyme CYP3A4 by components of grapefruit juice, is shown in Fig 2. Control nisoldipine bioavailability (F) was considered to be 3.9% on the basis of a previous report.37

The intestinal intrinsic clearance (CLGI,int) and hepatic intrinsic clearance (CLH,int) were calculated as follows in the absence of grapefruit juice. The CLH,int was determined based on the following equations:

\[
\begin{align*}
CL_{iv} &= Q_H \cdot CL_{H,int} / (Q_H + CL_{H,int}) \\
CL_{H,int} &= Q_H \cdot CL_{iv} / (Q_H - CL_{iv})
\end{align*}
\]

in which QH and CLiv are the hepatic blood flow and the total body clearance after intravenous administration, respectively. We assumed that CLGI,int did not contribute to the CLiv after intravenous administration of the drug.

The total clearance (CLoral) after oral administration was calculated as follows:

\[
CL_{oral} = \frac{Dose}{AUC}
\]
The bioavailability in the liver (F_{\text{Liver}}) and the absolute bioavailability (F) of the drug after oral intake were given as follows:

\[ F_{\text{Liver}} = \frac{Q_H}{Q_H + C_{\text{Liver,int}}} \] (3)

\[ F = \frac{C_{\text{Liver,int}}}{C_{\text{oral}}} = F_{\text{abs}} \cdot F_{\text{GI}} \cdot F_{\text{Liver}} \] (4)

If we assume F_{\text{abs}} = 1, the bioavailability in the intestine (F_{\text{GI}}) can be determined by use of F and F_{\text{Liver}}:

\[ F_{\text{GI}} = \frac{F}{F_{\text{Liver}}} \]

On the other hand, F_{\text{GI}} can be expressed as follows:

\[ F_{\text{GI}} = \frac{Q_{\text{GI}}}{Q_{\text{GI}} + C_{\text{GI,int}}} \] (5)

Thus, CL_{\text{GI,int}} can be determined by use of equations 1, 4, and 5. We can also determine the intestinal intrinsic clearance (CL_{\text{GI,int}}) in the presence of grapefruit juice by the same procedure.

The intestinal intrinsic clearance of felodipine (CL_{\text{GI,int}}) in the presence of grapefruit juice and that in the absence of grapefruit juice (CL_{\text{GI,int}}^\text{a}) can be determined as follows.

In the absence of grapefruit juice:

\[ CL_{\text{GI,int}} = V_{\text{max}}/K_m \] (6)

\[ V_{\text{max}} = k_p \cdot E_t \] (7)

In the presence of grapefruit juice:

\[ CL_{\text{GI,int}}^\text{a} = V_{\text{max}}^\text{a}/K_m \] (8)

\[ V_{\text{max}}^\text{a} = k_p^\text{a} \cdot E \] (9)

in which \( V_{\text{max}} \) (in \( \mu \text{mol/min} \)) and \( E_t \) (in \( \mu \text{mol} \)) are the maximum reaction velocity and the total active CYP3A4 content, respectively, in the absence of grapefruit juice. The \( V_{\text{max}}^\text{a} \) (in \( \mu \text{mol/min} \)) and \( E \) (in \( \mu \text{mol} \)) are those in the presence of grapefruit juice. \( K_m \) (in \( \text{mmol/L} \)) and \( k_p \) (in \( \text{min}^{-1} \)) are the Michaelis-Menten constant of the enzyme reaction and the metabolic rate constant, respectively. We assumed that these two parameters are unaffected in the presence of grapefruit juice and that only the active CYP3A4 content (E) is changed in the presence of grapefruit juice.

Thus the ratio (\( \varepsilon \)) of the active CYP3A4 contents in the presence and absence of grapefruit juice is given by the following:

\[ CL_{\text{GI,int}}/CL_{\text{GI,int}}^\text{a} = V_{\text{max}}^\text{a}/V_{\text{max}} = E/E_t = \varepsilon \] (10)

The small intestinal transit time of the solution was obtained by subtracting the gastric emptying time from colon arrival time.

The concentration–time profile (\( C_{\text{GFJ}} \)) of orally administered grapefruit juice in the intestinal tract is therefore assumed to follow the one-compartment model:

\[ C_{\text{GFJ}} = k_{\text{a}}/\left(k_{\text{a}} - k_{\text{e}}\right) \cdot \left[\exp(-k_{\text{e}} \cdot t) - \exp(-k_{\text{a}} \cdot t)\right] \] (11)

in which \( k_{\text{a}} \) (in \( \text{hr}^{-1} \)) and \( k_{\text{e}} \) (in \( \text{hr}^{-1} \)) are the inflow rate constant of grapefruit juice from the stomach to the small intestine and the outflow rate constant of grapefruit juice from the small intestine to the colon, respectively, and \( t \) (in hours) is the time since grapefruit juice ingestion. \( C_{\text{GFJ}} \) is an arbitrary value.

Grapefruit juice components are presumed to react specifically with CYP3A4 in intestinal cells, converting active CYP3A4 to inactive CYP3A4 with a rate constant of \( K \) (in arbitrary units \( \text{[AU]}^{-1} \cdot \text{hr}^{-1} \)). Furthermore, it is assumed that CYP3A4 is synthesized at a constant rate (\( K_s \); in \( \text{mol} \cdot \text{hr}^{-1} \)) and is eliminated with the first-order rate constant (\( k \); in \( \text{hr}^{-1} \)). Therefore the total CYP3A4 content (\( E_t \); in \( \text{mol} \)) should be given by \( K_s/k \) for steady state. The time-dependent changes of the active CYP3A4 content (\( E \); in \( \text{mol} \)) and the inactive CYP3A4 content in the presence of grapefruit juice components (\( E_c \); in \( \text{mol} \)) are given by the following equations:

\[ \frac{dE}{dt} = -K \cdot C_{\text{GFJ}} \cdot E - k \cdot E + K_s \] (12)

\[ \frac{dE_c}{dt} = K \cdot C_{\text{GFJ}} \cdot E - k \cdot E_c \] (13)

In these equations, it is assumed that the elimination rate constant of \( E_c \) is the same as that of \( E \). Nonlinear least-squares regression analysis was conducted with the program MULTI (RUNGE) and \( K \) and \( k \) were estimated to be as follows (estimated value ± SD):

\[ K = 1.59 \pm 0.406 \]
\[ k = 0.0237 ± 0.00412 \]
\[ \text{AIC} = -6.18 \]

in which AIC is an information criterion. Analysis on the basis of this assumption shows a better agreement between prediction and observation than an alternative analysis based on different elimination constants for \( E \) and \( E_c \) (\( k \) and \( k^\prime \), respectively; analysis not shown), and this allowed us to decrease the number of parameters by using a single elimination constant (\( k \)).

Data analysis

The pharmacokinetic parameters for gastrointestinal transit of grapefruit juice (\( k_s \) and \( k_e \)) were estimated by use of reported data on the behavior of orally administered grapefruit juice in the stomach and intestine.
The $k_a$ value was determined as the reciprocal of gastric emptying time (0.30 hours); that is, $3.33$ (in h$^{-1}$). The intestinal transit time (4.10 hours) was calculated from the difference between the gastric emptying time (0.30 hours) and the arrival time at the colon (4.40 hours). The $k_e$ value was determined as the reciprocal of the intestinal transit time, 0.244 (in h$^{-1}$).

Fig 3. Mean changes in diastolic blood pressure, mean blood pressure, systolic blood pressure, and heart rate against time after nisoldipine administration in the control group (A), G0 (B), G14 (C), G38 (D), G72 (E), and G96 (F). On the study days the subjects were treated according to the schedule in Fig 1. Each point represents the mean value ± SEM of eight volunteers for the control group to G72 and seven volunteers for G96. Significant differences from the control group were identified by the Student paired $t$ test (*$P < .05$; **$P < .01$). Symbols represent diastolic blood pressure (open triangles), mean blood pressure (open squares), systolic blood pressure (open circles), and heart rate (solid circles).
The subjects were treated according to the schedule as shown in Fig 1. Each point represents the mean ± SEM of eight volunteers for control to G72 and seven volunteers for G96.

Significant differences from control were identified by the Student paired t test: *P < .05; **P < .01.

### Table II. Pharmacokinetic parameters of nisoldipine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC ($\times 10^{-6}$ % of dose · h/mL)</th>
<th>$C_{\text{max}}$ ($\times 10^{-6}$ % of dose · h/mL)</th>
<th>$t_{1/2}$ (h)</th>
<th>$t_{\text{max}}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78.1 ± 11</td>
<td>24.0 ± 3.4</td>
<td>1.86 ± 0.33</td>
<td>1.50 ± 0.16</td>
</tr>
<tr>
<td>G0</td>
<td>321 ± 46***</td>
<td>118 ± 19***</td>
<td>1.42 ± 0.11</td>
<td>1.69 ± 0.33</td>
</tr>
<tr>
<td>G14</td>
<td>181 ± 23***</td>
<td>71.0 ± 12*</td>
<td>1.70 ± 0.19</td>
<td>1.00 ± 0.13</td>
</tr>
<tr>
<td>G38</td>
<td>132 ± 19**</td>
<td>45.0 ± 7.0</td>
<td>1.69 ± 0.20</td>
<td>1.50 ± 0.41</td>
</tr>
<tr>
<td>G72</td>
<td>107 ± 11***</td>
<td>30.0 ± 4.0</td>
<td>2.04 ± 0.21</td>
<td>0.875 ± 0.13</td>
</tr>
<tr>
<td>G96</td>
<td>108 ± 18</td>
<td>40.0 ± 13</td>
<td>1.54 ± 0.20</td>
<td>1.29 ± 0.31</td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration–time curve; $C_{\text{max}}$, maximum plasma concentrations; $t_{1/2}$, terminal half-life; $t_{\text{max}}$, time to reach $C_{\text{max}}$.

In the study, we wished to estimate the time-dependent change of nisoldipine AUC in the presence of grapefruit juice. However, AUC is the sum of the area under the plasma concentration curve from administration to infinity. We therefore used $t_{\text{max}}$ at the time of $C_{\text{max}}$ as a measure of the interaction effectiveness with grapefruit juice. If $t$ is real time after grapefruit juice ingestion, the effect of grapefruit juice ingestion to the active CYP3A4 is that the ratio of the active CYP3A4 decreases just before the grapefruit juice ingestion by the analysis. This result is unnatural and difficult to analyze. We therefore took $t$ as “$t_{\text{max}}$ plus real time after ingestion of grapefruit juice.”

On the basis of the parameter values thus obtained, we used the model to simulate the time-dependent changes of the active CYP3A4 content ratio ($\varepsilon$), and the increase of nisoldipine AUC was calculated by use of the following equation:

\[
AUC'/AUC = (Dose \cdot F/CL_{\text{IV}})/(Dose \cdot F/CL_{\text{IV}})
\]

\[
AUC'/AUC = F'GI/FGI
\]

\[
AUC'/AUC = |Q_{GI}(Q_{GI} + CL_{GI,int})|/|Q_{GI}(Q_{GI} + CL_{GI,int})|
\]

\[
AUC'/AUC = (R + 1)/(R + \varepsilon)
\]

in which $R$ is $Q_{GI}/CL_{GI,int}$.

### RESULTS

#### Pharmacodynamics

**Hemodynamic effects and adverse events based on grapefruit juice–nisoldipine interaction.** There were no significant differences in baseline blood pressure or heart rate on the 6 experimental days (Fig 3). As shown in Fig 3 and Table I, grapefruit juice ingestion after drug administration did not affect heart rate or the AUE(0-8) of heart rate. On the other hand, significant decreases of systolic blood pressure and diastolic blood pressure were found until 8 hours after drug intake between the control experiment and the simultaneous administration.
of grapefruit juice and drug (G0) (Fig 3). A significant decrease of AUE(0-8) of systolic blood pressure was found (Table I). Adverse events were spontaneously reported during each treatment. Headaches were reported by three persons in G0, two persons in G14, one person in G38, and no subjects in G72 and G96.

Pharmacokinetics

Nisoldipine. The mean plasma concentration–time profiles of nisoldipine are shown in Fig 4, and the mean values of the pharmacokinetic parameters are given in Table II. Compared with the control group, the C_{max} value of nisoldipine was significantly increased after grapefruit juice and drug treatment.
Fig 5. Plasma concentration profiles of BAY r-9425, BAY r-9590, and BAY o-3199 in the control group (A), G0 (B), G14 (C), G38 (D), G72 (E), and G96 (F). On the study days the subjects were treated according to the schedule in Fig 1. Each point represents the mean value ± SEM of eight volunteers for the control group to G72 and seven volunteers for G96. Significant differences from the control group were identified by the Student paired t test (*P < .05; **P < .01; ***P < .001).
juice in G0 and G14, and the plasma concentration was significantly increased at each time in G0 to G72 compared with the control group, thereby indicating that the effect of grapefruit juice decreased time dependently and lasted for at least 3 days after intake. As shown in Table II, AUC in G0, G14, G38, G72, and G96 was increased 4, 2.3, 1.7, and 1.4 times, respectively, compared with the control group (experiment 1). C\textsubscript{max} values in G0, G14,
G38, G72, and G96 were increased 5, 3, 2, and 1.2 times, respectively, compared with the control group. On the other hand, there were no significant differences in terminal t1/2 or tmax values. In accordance with the systolic blood pressure decrease and reduction of adverse events, the effect of grapefruit juice decreased time dependently.

**BAY r-9425, BAY r-9590, and BAY o-3199.** As shown in Fig 5, the plasma concentrations of nisoldipine metabolites (BAY r-9425, BAY r-9590, and BAY o-3199) were significantly increased at each time after grapefruit juice in G0 and G14 compared with the control group. As shown in Fig 6, AUC of values of BAY r-9425 and BAY o-3199 in G0 and G14 were increased compared with the control group, and AUC values of BAY r-9590 in G0, G14, G38, and G72 were also increased compared with the control group.

**Estimation of the reaction rate constant (K) between grapefruit juice and CYP3A4 and the elimination rate constant (k) of CYP3A4**

On the basis of the irreversible enzyme inhibition model (Fig 2), pharmacokinetic data (ε value-time profiles) of nisoldipine (Table III) after single or repeated ingestion of grapefruit juice were fitted to the model with a nonlinear least-squares regression analysis program to estimate K and k. The estimated values of K and k were 1.59 ± 0.406 AU⁻¹ · h⁻¹ and 0.0237 ± 0.00412 h⁻¹, respectively. The simulation curves and the observed values of active CYP3A4 ratio (ε) and change of nisoldipine AUC after single or repeated ingestion of grapefruit juice are shown in Fig 7. Good agreement was found between the observed and predicted values.

**DISCUSSION**

The plasma concentrations of various drugs are increased after grapefruit juice ingestion.²⁴ These drugs are predominantly metabolized by CYP3A4, CYP3A5, or both. This clinically important grapefruit juice–drug interaction appeared specifically after oral administration of felodipine, nifedipine, midazolam, and cyclosporine, but not after intravenous administration, and was suggested to be the result of competitive inhibition of CYP3A4 function or down-regulation of CYP3A4²⁶,²⁷ or of competitive inhibition of P-glycoprotein function.²⁸ Quantitative evaluation of this grapefruit juice–drug interaction is required for the proper clinical management of patients. We have therefore constructed a pharmacokinetic model with use of felodipine extended release ER data.³,⁴ This model (Fig 2) was constructed on the assumption that CYP3A4 is down-regulated irreversibly by components of grapefruit juice. Lown et al²⁶ reported that the CYP3A4 protein content was decreased by grapefruit juice at the level of Western blotting, supporting the idea that the enhanced oral availability of CYP3A4 substrates induced by ingestion of grapefruit juice is based on downregulation of CYP3A4. Based on felodipine–grapefruit juice interaction, the grapefruit juice–CYP3A4 reaction constant (K) and CYP3A4 elimination constant (k) are 0.922 ± 0.0688 AU⁻¹ · h⁻¹ and 0.0849 ± 0.00913 h⁻¹, respectively.³³ Therefore, the terminal t½ of CYP3A4 is calculated as 8 hours. The simulation showed that twice the amount of grapefruit juice would produce almost the maximum effect on felodipine pharmacokinetics, and it would take at least 3 days after the last grapefruit juice ingestion for the level of active CYP3A4 to recover fully. In this clinical study of grapefruit juice–nisoldipine interaction, simultaneous intake gave the highest incidence of adverse effects and the greatest AUC increase, and increasing the interval after grapefruit juice caused both parameters to return to control levels (Fig 6 and Table II). The changes in levels

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**Fig 7.** The active CYP3A4 ratio (ε; A) and AUC ratio (B) before and after intake of grapefruit juice based interaction between grapefruit juice and nisoldipine. *Solid circles* represent the experimental data; *solid lines* represent the fitting curves according to the irreversible enzyme inhibition model (K = 1.596 ± 0.388 AU⁻¹ · h⁻¹; k = 0.0230 ± 0.00376 h⁻¹).
of metabolites (BAY r-9425, BAY r-9590, and BAY o-3199) were also consistent with this pattern (Fig 6).

We fitted the data (Table III) for nisoldipine to our model by nonlinear least-squares regression analysis to estimate the reaction rate constant between grapefruit juice and CYP3A4 (K) and the elimination rate constant of CYP3A4 (k). Good agreement was obtained between observed data and values predicted by the model, as shown in Fig 7. The estimated parameter values were 1.60 ± 0.388 h⁻¹ for K and 0.0230 ± 0.00376 h⁻¹ for k. In developing the pharmacokinetic model (Fig 7), we also analyzed the elimination rate constant of active CYP3A4 (k) independently of that of inactive CYP3A4 (k') but found that this gave no improvement over the use of a single rate constant. Therefore, in this analysis, we assumed that their elimination rate constants are the same. It was desirable to minimize the number of parameters to be estimated in this study because we had only 5 points as observed data.

In this study, the terminal t₁⁄₂ of CYP3A4 was calculated to be 30 hours, which is 3 to 4 times greater than the previously calculated terminal t₁⁄₂ value (8 hours). We thought this might be because of (1) quantitative or qualitative differences of grapefruit juice components depending on the production batch, (2) different races of subjects, (3) different times of data collection between the data of Lundahl et al³ and ours (Lundahl et al³ gave grapefruit juice 0, 1, 4, 10, or 24 hours before the tablet, whereas we gave grapefruit juice three times a day for 7 days, finishing 14, 38, 72, or 96 hours before the tablet), or (4) the dosage form (Lundahl et al³ used felodipine ER, while we used nisoldipine plain tablet). Nisoldipine may be a better indicator of grapefruit juice–drug interaction because the AUC increase of nisoldipine is higher than that of felodipine ER. The increase in AUC after grapefruit juice was significantly less for a felodipine ER than that of felodipine ER. The increase in AUC after grapefruit juice ingestion was almost eliminated and AUC returned to the control level by 3 days after the last grapefruit juice intake (Fig 6).

In this study, the grapefruit juice absorption constant (kₐ) was assumed to be the same as the gastrointestinal solution transit, but it will be necessary to examine the effect of food on grapefruit juice gastrointestinal transit. Furthermore, we considered only the effect of grapefruit juice on the CYP3A4 content in the intestine. It is known that P-glycoprotein in the intestine also contributes to the low bioavailability of some drugs, but the contributions of the two mechanisms (CYP3A4 and P-glycoprotein) seem to differ from case to case. Therefore it will also be necessary to consider quantitatively the effect of P-glycoprotein on the absorption of drugs such as cyclosporine and tacrolimus. To date, dihydroxybergamottin, bergamottin, GF-I-1 (FC726), and GF-I-4 have been identified as specific inhibitors of CYP3A4 in grapefruit juice. We recently found that the furanocoumarin derivatives in grapefruit juice also inhibit P-glycoprotein function in Caco-2 cells (Matsuo H, et al, unpublished data, September 1999); these inhibitors may be useful in evaluating the contribution ratio of the two mechanisms. It may be necessary to modify the present model of grapefruit juice–drug interaction.

It has been reported that grapefruit juice had no effect on the pharmacokinetics of each enantiomer of nitrendipine. Thus there may be no effect of grapefruit juice on the pharmacokinetics of each enantiomer of nisoldipine. However, we did not study the differences in the enantiomers of the active drug between patients. There may be differences in the enantiomers of the active drug between patients. Further studies will be necessary.

In conclusion, we conducted a clinical study of grapefruit juice–nisoldipine interaction and analyzed the results according to a pharmacokinetic model (Fig 2). As expected from the previous simulation study, the AUC value recovered to the control level more than 3 days after the final grapefruit juice ingestion. To prevent grapefruit juice–nisoldipine interaction, it is therefore necessary to withhold grapefruit juice for at least 3 days before administration of the drug.

References
4. Lundahl J, Regardh CG, Edgar B, Johnsson G. The inter-


