Effect of a Single Glass of Grapefruit Juice on the Apparent Oral Bioavailability of the Dihydropyridine Calcium Channel Antagonist, Azelnidipine, in Healthy Japanese Volunteers

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There are many reports on grapefruit juice (GFJ) increasing the apparent oral bioavailability of several clinically important drugs metabolized by the most abundant isoform of cytochrome P450, i.e. CYP 3A4. Azelnidipine (Calblock®) is a long-lasting 1,4-dihydropyridine calcium antagonist currently used in the treatment of hypertension in Japan. In a drug interaction study using human liver microsomes, several CYP3A4 inhibitors and substrates inhibited the oxidative metabolism of azelnidipine to the same extent as nifedipine and felodipine. In order to evaluate the possible interaction of azelnidipine with GFJ in humans, a randomized, two-way crossover study was conducted in eight Japanese healthy volunteers.

A single oral dose of 8 mg azelnidipine was administered orally with either 250 mL water or GFJ after overnight fasting. Blood samples were drawn periodically up to 24 hours after dosing. Plasma concentrations of azelnidipine were measured by liquid chromatography-tandem mass spectrometry (LC/APCI-MS/MS).

Concomitant administration of azelnidipine with GFJ increased the mean C of azelnidipine by 2.5-fold and the AUC by 3.3-fold compared with water; moreover, the time to reach C (tmax) and the mean residence time (MRT) were slightly delayed. No serious adverse events were observed except one subject described mild symptoms of drug-related headache and flushing accompanied with orthostatic hypotension at 4 hrs after administration in the GFJ phase.

The results demonstrated the pharmacokinetic interaction between azelnidipine and a single glass of GFJ.

Key words: azelnidipine, grapefruit juice, CYP3A4, drug interaction

Introduction

It is well known that more than half of the currently available drugs are metabolized by cytochrome P450 3A4 (CYP3A4), a major isoform in human1). Recently it has been reported that CYP3A4 expressed in the apical lumen of the small intestine participates in presystemic clearance of exogenous substances including drugs as well as that expressed in the hepatocytes. Thereby, when a drug mainly metabolized by CYP3A4 in the small intestine is administered orally, the enzyme plays a significant role in regulating the extent and rate of bioavailability of the drug.

Azelnidipine (Calblock®) is a L-type calcium channel selective antagonist, which is categorized as third generation type-B9). The remarkable characteristics of azelnidipine that differentiate it from other analogues are 1) the lower possibility to cause sinus bradycardia in patients and, 2) longer-lasting pharmacological effects even with a once daily dose of 8-16 mg9).

Metabolism of azelnidipine was inhibited by several CYP3A4 inhibitors and substrates to the same extent as nifedipine and felodipine in a previous in vitro drug interaction study using human liver microsomes4). Numerous reports have shown that concomitant ingestion of grapefruit juice (GFJ) results in an increase in the plasma concentrations of several drugs in clinical use5-13). In our previous

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study, we clarified that prior GFJ ingestion significantly alters the pharmacokinetic behavior of atorvastatin, namely an increase of systemic exposure of both parent acid and lactone forms accompanied with a decrease of 2-hydroxyl metabolites. In contrast, no significant changes were detected in any pharmacokinetic parameters of pravastatin examined when taken with GFJ.

This increase of systemic exposure is considered to be due to the mechanism-based inhibition of CYP 3A4 activities in the small intestine by subsequent reduction in presystemic metabolic clearance. Indeed after intravenous administration, the plasma levels of these drugs were not altered by GFJ ingestion.

Concomitant ingestion of GFJ with dihydropyridine calcium antagonists was reported to induce several overdose-like vascular-related adverse events, such as headache, flushing and accelerating heart rate, even though the studies were performed with healthy adults.

There are also some reports of an increase in the risk of side effects of anxiolytic agents administered with GFJ. In the most recently reported cases, however, the effects were mild and not considered clinically significant. Caution should be paid especially for drugs with a small therapeutic range and/or more likely to induce a pharmacological over-response when taking GFJ.

In the present study, we investigated the extent of drug interaction between azelnidipine and GFJ in Japanese healthy male volunteers.

Methods

1. Subjects

Eight healthy male volunteers (age range: 23–40 years; weight range: 53.0–73.0 kg) participated in the study as shown in Table 1. Each subject was ascertained to be in good health by a medical history, a clinical examination and routine laboratory tests. The subjects were not taking any other continuous medication, and all of them were nonsmokers. They were not allowed to consume any grapefruit products during the study period from 1 week before the day of first dosing to the day after discharge from the hospital. This study was conducted between September 11, 1998 and October 13, 1998 at the Medical Corporation Keiyu-Kai, Obara Hospital, Tokyo, Japan, in compliance with good clinical practice and other related statutes. The subjects gave their written informed consent before entering the study. Approval was obtained from the Institutional Review Board of the medical institution in which the study was conducted.

2. Study design

The study was performed with a randomized, open-labeled two-way crossover design with an interval of 1 week. The volunteers ingested 250 mL of 100% grapefruit juice (Tropicana™ Homemade Style, Kirin Beverage Corp., Tokyo, Japan: Lot No. # 0293) or water (250 mL) on the day of each treatment at 9:00 a.m. with one 8 mg tablet of azelnidipine (Sankyo Co., Ltd., Tokyo, Japan).

The subjects fasted for 14 hours before administration of azelnidipine, and a standardized meal and light snack were served at 4 hours and 7 hours after taking the test substance, respectively. The subjects were not allowed to smoke cigarettes, to drink alcohol, coffee or tea, or to take St. John’s Wart during the study from 1 week before admission to the hospital to the day after discharge from the hospital.

3. Safety evaluation

Safety assessment was performed throughout the study including vital signs (body temperature, systolic and diastolic blood pressure, and pulse rate), electrocardiography, hematology, clinical chemistry and urinalysis as well as clinical observations (subjective and/or objective symptoms). Adverse events were monitored and recorded appropriately.
4. Blood sampling

Venous blood (10 mL) was collected from the brachial vein of each subject just before administration of azelnidipine and 1/2, 1, 2, 3, 4, 6, 8, 12 and 24 hrs after administration. Blood was sampled into a siliconized Venoject tube and the plasma portion was separated within 30 minutes and stored at -20°C until analysis.

5. Determination of azelnidipine in plasma

Plasma concentrations of azelnidipine were determined by a pre-validated liquid chromatography-tandem mass spectrometry (LC/APCI-MS/MS TSQ7000, Finnigan MAT Instruments, Inc.) at BML Inc. (Tokyo, Japan) in compliance with good laboratory practice. The lower limit of quantification was 0.6 ng/mL. The intra- and inter-assay accuracy (RE) ranged from -8.6% to +6.1% for three different QC samples and met the predefined analytical criteria throughout the analysis.

6. Pharmacokinetic analysis

The pharmacokinetics of azelnidipine was characterized by a series of non-compartmental parameters, i.e. peak plasma concentration (Cmax), time to reach Cmax (tmax), mean residence time (MRT), elimination half-life (t1/2), area under the plasma concentration-time curve (AUC0-24h) and apparent oral clearance (CL/F). These parameters were calculated based on the individual plasma concentrations using commercially available software, WinNonlin™ (ver. 1.5, Pharsight).

7. Statistical analysis

Plasma concentrations at each time point are expressed as arithmetic mean±SD. A geometric mean and its 95% confidence interval were calculated for the Cmax and AUC0-24h. For the other pharmacokinetic parameters (tmax, MRT, t1/2 and CL/F), an arithmetic mean and its 95% confidence interval (CI) were calculated. Analysis of variance for two-by-two crossover fashion was conducted for each pharmacokinetic parameter to confirm the differences between the GFJ and water treatments, and subjects and dosing periods, respectively. The sample size of the study (n=8) was determined by referring to the AUC data in the previous phase I study. We assumed 35% of the inter-subject variation (%CV) in mean AUC0-24h and 2-fold alteration should be considered as clinically relevant change. The overall type-I error (α) and the power of a test (1-β) are set at 0.05 and 0.90, respectively. Safety data (clinical observations, physical examinations and clinical laboratory tests) were analyzed by the Student's t-test (2-tailed) for quantitative variables or by the Wilcoxon signed-rank test for categorical variables. The differences were considered to be statistically significant when the p-value was less than 0.05.

Results

Mean plasma azelnidipine concentration-time curves for each treatment group are shown in Figure 1, and the details of the non-compartmental pharmacokinetic parameters are summarized in Table 2.

Concomitant ingestion with GFJ increased the systemic exposure of azelnidipine as follows. The geometric means of the Cmax of azelnidipine in the GFJ treatment and water treatment were 15.7 ng/mL (95% CI: 12.8-19.2) and 6.3 ng/mL (95% CI: 5.6-7.0), respectively. The Cmax showed a 2.5-fold increase (95% CI: 2.12-2.95; p<0.01) by GFJ.

The geometric means of the AUC0-24h in the GFJ treatment and water treatment were 147.9 ng•hr/mL (95% CI: 120.6-181.4) and 45.1 ng•hr/mL (95% CI: 39.0-52.0), respectively. The AUC0-24h showed a 3.3-fold increase (95% CI: 2.89-3.73; p<0.01) by GFJ.

The arithmetic means of the tmax in the GFJ treatment and water treatment were 2.1 hr (95% CI: 1.8-2.4) and 3.9 hr (95% CI: 3.0-4.7), respectively and the difference was statistically significant (p<0.01). However, there was no statistically significant difference in the t1/2 between the GFJ group and water group.

No serious drug-related adverse events were observed throughout the study. One subject (No. 1) described mild and possibly drug-related symptoms such as headache and facial flushing at 4 hrs after
Fig. 1 Plasma concentrations of azelnidipine after an oral administration of 8 mg azelnidipine tablet with either a single glass of water (○) or grapefruit juice (●). Data are expressed as mean±SD.

azelnidipine administration with GFJ. This subject also showed a temporal orthostatic hypotension (Fig. 2), which disappeared soon without any medical treatment and was not reproducible.

Individual systemic exposure, i.e. AUC0–24h and Cmax of azelnidipine at the water or GFJ phase are shown in Figure 3. The inter-individual variation in AUC0–24h and Cmax was about two-fold and a remarkable difference between the two treatments was not found. The Cmax and AUC of subject No. 1 at the GFJ phase (depicted as bold lines) were 9.6 ng/mL and 92.8 ng·hr/mL, respectively. These values were the lowest in magnitude among eight subjects.

Discussion

Numerous reports state that concomitant ingestion with GFJ results in elevation of plasma concentrations of several drugs and subsequent clinical relevant changes in their efficacy and toxicity5–13). CYP3A4, the most abundant cytochrome P450 isoform, is commonly related to the metabolism of these drugs. The increase in systemic exposure of such drugs has been recognized well due to the mechanism-based inhibition of CYP3A4 expressed at the small intestine by several kinds of furocoumarin analogues, such as 6',7'-dihydroxybergamottin and bergamottin, in GFJ and subsequent elevation of intestinal bioavailability20,21). As these inhibitory constituents are not absorbed from the gastrointestinal tract and are localized there at a substantially high level, the action is just limited to CYP3A4 expressed in the gut to eventually increase the intestinal first-pass bioavailability (f胃肠). The irreversible nature of inhibition prolongs the effect beyond a few days, which is governed by the enzyme turnover rate corresponding to a half-life of 8.16 hrs22). In particular, calcium antagonists are the most frequently reported for food-drug interactions with GFJ. Likewise, an increase in systemic exposure of azelnidipine, a dihydropyridine calcium antagonist, was observed with ingestion of GFJ in the present study.

In this study, a remarkable increase of 2.5 fold for Cmax and 3.3 fold for AUC0–24h was found after concomitant ingestion of GFJ and azelnidipine. In previously reported GJF interaction studies with calcium antagonists such as felodipine, nisoldipine, nifedipine and amlodipine under similar study designs, the magnitude of systemic exposure (AUC) increase by GFJ were quite different: 2.84 fold for felodipine6), 4.06

### Table 2 Non-compartmental pharmacokinetic parameters after oral administration of an 8 mg azelnidipine tablet with a single glass of water or grapefruit juice in 8 healthy subjects.

<table>
<thead>
<tr>
<th>Parameter1)</th>
<th>Water (control)</th>
<th>Grapefruit juice</th>
<th>Change from control2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>6.3 (5.6, 7.0)</td>
<td>15.7 (12.8, 19.2)</td>
<td>2.5* (2.1, 3.0)</td>
</tr>
<tr>
<td>AUC0–24h (ng·hr/mL)</td>
<td>45.1 (39.0, 52.0)</td>
<td>147.9 (120.6, 181.4)</td>
<td>3.3* (2.9, 3.7)</td>
</tr>
<tr>
<td>tmax (hr)</td>
<td>2.1 (1.8, 2.4)</td>
<td>3.9 (3.0, 4.7)</td>
<td>1.8* (0.9, 2.6)</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>6.3 (5.6, 7.1)</td>
<td>8.3 (7.9, 8.6)</td>
<td>1.9* (1.1, 2.7)</td>
</tr>
<tr>
<td>t1/2 (hr)</td>
<td>6.5 (4.1, 8.8)</td>
<td>7.7 (6.8, 8.5)</td>
<td>1.2 (0.7, 3.1)</td>
</tr>
<tr>
<td>CL/F (mL/min)</td>
<td>2998 (2585, 3412)</td>
<td>926 (723, 1129)</td>
<td>−2072* (−2417, −1727)</td>
</tr>
</tbody>
</table>

1) Cmax and AUC0–24h are expressed as geometric means, while tmax, MRT, t1/2 and CL/F are arithmetic means. Values in parentheses represent the lower and upper 95% confidence intervals, respectively.

2) Changes from control for Cmax and AUC0–24h indicate the mean ratio of grapefruit juice treatment to water treatment. Those for tmax, MRT, t1/2 and CL/F are expressed as the mean difference between grapefruit juice and water treatments. An asterisk represents a significant difference found between the two treatment groups with p<0.01.
fold for nisoldipine\textsuperscript{17}, 1.34 fold for nifedipine\textsuperscript{6} and 1.15 fold for amlodipine\textsuperscript{23}. Based on the criteria proposed by Bailey et al\textsuperscript{7}, azelnidipine might be categorized as highly susceptible against GFJ like felodipine and nisoldipine. The $t_{\text{max}}$ and MRT were also delayed by GFJ ingestion, suggesting that GFJ affected not only the extent of bioavailability of azelnidipine but also the retention of azelnidipine in the intestinal tract.

The mean $t_{1/2}$ of azelnidipine seemed to be slightly prolonged from 6.5 hr in the control group to 7.7 hr in the GFJ group. However, there was no statistically significance. In contrast, the apparent oral clearance (CL/F) was significantly decreased by GFJ ingestion. Similar findings have been reported for other dihydropyridine calcium antagonists, such as in the felodipine-GFJ interaction study\textsuperscript{14}). These results suggested that the concomitant effect of GFJ might be limited to the intestine and GFJ did not affect the liver. The inter-individual variability of $C_{\text{max}}$ and AUC\textsubscript{0–24h} in this study (corrected for body weight) was comparable between two treatment phases and was somewhat smaller than those of three calcium antagonists under extensive CYP3A4 metabolism\textsuperscript{24}).

Following concomitant administration with GFJ, different magnitudes of change in the AUC and $C_{\text{max}}$ were observed among various drugs\textsuperscript{6}, and in particular there was a clear relationship with the first pass bioavailability of several dihydropyridine calcium antagonists\textsuperscript{7}. The absolute bioavailabilities in healthy subjects were as follows: less than 10\% for nisoldipine\textsuperscript{16}, 13–21\% for felodipine\textsuperscript{28}, 50–60\% for nifedipine\textsuperscript{29} and 81\% for amlodipine\textsuperscript{27}. 

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**Fig. 2** Time-course alterations in SBP and DBP in supine position of eight healthy subjects after oral administration of an 8 mg azelnidipine tablet with either a single glass of water (A) or grapefruit juice (B)

The bold lines represent supine blood pressures of subject No. 1 who described mild drug-related symptoms at the grapefruit juice phase.

**Fig. 3** Individual $C_{\text{max}}$ (A) and AUC\textsubscript{0–24h} (B) after oral administration of an 8 mg azelnidipine tablet with either a single glass of water or grapefruit juice (GFJ)

The bold lines represent subject No. 1 who described mild drug-related symptoms at the grapefruit juice phase.
It is noteworthy that a drug with a lower absolute bioavailability tends to show a greater increase in the exposure after concomitant ingestion of GFJ. However, it is also known that the content of CYP3A4 and its distribution in the intestine are variable among subjects. Considering 30-fold difference in activity and content of CYP3A4 among individuals, we should select the appropriate drug for each patient based on the balance of efficacy and any adverse symptoms during therapy.

The first pass metabolism of azelnidipine via CYP3A4 expressed at the intestine might be substantially inhibited by GFJ ingestion. No serious drug-related adverse effects were observed in this study even though the magnitude of systemic exposure increase by GFJ ingestion was rather high compared with other dihydropyridine calcium antagonists. One subject (No. 1) described headache and facial flushing at 4 hrs after drug administration with GFJ. A temporal and not reproducible orthostatic hypotension was also observed accompanied with these possibly drug-related symptoms. Interestingly, the systemic exposure of this subject at the GFJ phase was not as high compared with the other seven subjects (Fig. 3). Considering the hypotensive background of this subject (Table 1), the latter symptom might be caused by baroreceptor reflex impairment commonly occurring in such a population. In a previous felodipine and GFJ interaction study, both flushing and headache were observed in or reported by all subjects due to vasodilatation, although these symptoms were mild and did not lead to the withdrawal of any subjects from the study.

An in vitro study showed that azelnidipine reversibly blocks voltage-dependent Ca²⁺ influx through L-type calcium channels in the cell membrane. Azelnidipine exerts a long duration of pharmacological action due to its high lipophilicity, which the first or second generation of calcium channel antagonists do not exhibit. The elimination half-life of azelnidipine in healthy subjects marginally deviates among the reported studies; the summarized average ranges from 7 to 21 hrs, which is nevertheless longer than those of commonly used calcium antagonists except for amlodipine. The temporal orthostatic hypotension observed in subject No. 1 did not accompany high systemic exposure of drug, however, there is a concern that the concomitant intake of azelnidipine with GFJ chronically may cause significant elevation of the plasma level and a drug-related adverse reaction in patients. Consequently, an appropriate warning should be given for patients not taking GFJ during azelnidipine therapy.

In this study, we investigated the effect of a single glass of GFJ on the oral bioavailability of azelnidipine. Considering the pharmacokinetic and pharmacodynamic characteristics of azelnidipine, the possibility of any drug-drug interaction with concomitantly prescribed CYP3A4 related drugs should also be investigated.

Note: A part of this study was previously described in the package insert of Calblock® (Sankyo).

References

12) Ducharme MP, Provenzano R, Dehoorne-Smith M, Edwards


