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# Publication bias on clinical studies of pharmacokinetic interactions between felodipine and grapefruit juice

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Plasma concentrations of a variety of drugs are known to be increased by concomitant administration of grapefruit juice (GFJ) when the drugs are administered orally. Dihydropyridine Ca channel blockers, that form one of the major categories of antihypertensive, have been studied for interactions for the longest time in research history. Especially, felodipine has been the most studied dihydropyridine drug. Although a lot of clinical research has been performed on the pharmacokinetic variations of felodipine, there has been no adequate systematic study. Therefore, publications related to felodipine-GFJ interactions were integrated and analyzed with statistical procedures of meta-analysis to characterize these clinical studies. Furthermore, funnel plots were created to validate publication bias in the data. Integration of AUC values on GFJ-administered and control groups in 12 publications revealed that felodipine is apparently affected by interaction. However, publication bias was observed in the funnel plots, and null hypothesis of no bias was rejected by Begg's test. These findings suggest that the pharmacokinetic interactions with GFJ might be overrated in the fundamental trial stage.

## 1. Introduction

Plasma concentrations of various categories of drugs such as calcium channel antagonists (Bailey et al. 1991, 1993b; Soons et al. 1991; Josefsson et al. 1996; Sugawara 1996; Hashimoto et al. 1998; Fuhr et al. 1998; Uno et al. 2000; Yajima et al. 2003; Hirashima et al. 2006; Ohnishi et al. 2006), anti-malarial agents (van Agtmael et al. 1999a, b), benzodiazepines sedative-hypnotic agents (Hukkinen et al. 1995; Kupferschmidt et al. 1995; Christensen et al. 2002), and antianxiety agents (Lilja et al. 1998; Lee et al. 1999) were reported to increase following concomitant intake of grapefruit juice (GFJ). These findings can be traced to reports from 1989 and 1991 in which pharmacokinetic interactions between GFJ and dihydropyridine drugs such as nifedipine and felodipine were demonstrated (Bailey et al. 1989, 1991). Since then many clinical studies on GFJ interactions with a variety of medicines have been performed. These interactions have been confirmed to be attributable to the inhibition of intestinal CYP3A with furanocoumarin derivatives such as bergamottin and 6′,7′-dihydroxybergamottin present in GFJ (Lown et al. 1994; Schmiedlin-Ren et al. 1997; Fukuda et al. 2000; Wangensteen et al. 2003). CYP3A is an important drug oxidation enzyme in human small intestine (Obach et al. 2001). This enzyme blocks the intestinal absorption of small molecule xenobiotics from drugs, food and beverage components. Intake of GFJ reduces the enzymatic barrier function in the intestine resulting in increased bioavailabilities of CYP3A substrates (Lown et al. 1997). The extent of the increase has been found to be related to the physicochemical features, such as lipophilicity and protein-binding capacity of GFJ-interaction prone drugs (Ohnishi et al. 2006; Uesawa and Mohri 2008b). On the other hand, the potency of GFJ interactions varies greatly among studies even when the same drug is investigated (Uesawa and Mohri 2008a).

Presently, felodipine is the drug that is investigated the most for interactions in clinical studies. This is because it exhibits high sensitivity among dihydropyridine drugs, and it is likely being used heavily to characterize GFJ-drug interactions. However, systematic integration of these studies has never been reported. Therefore, we attempted to perform a meta-analysis of publications on felodipine-GFJ interactions to characterize the studies.

## 2. Investigations, results and discussion

### *2.1. Adopted publications*

Twelve publications for felodipine-GFJ interactions in human were extracted from the literature based on our criteria mentioned in the experimental section (Bailey et al. 1991; Edgar et al. 1992; Bailey et al. 1993a, 1995, 1996, 1998, 2000, 2003; Lundahl et al. 1995, 1997; Lown et al. 1997; Goosen et al. 2004). The published year, the first author's name, number of subjects, felodipine dosage, area under the plasma concentration-time curve (AUC) values with units, AUC values with units arranged as nmol·h/L/mg dosage, standard deviations, and felodipine AUC ratio (the AUC of the GFJ group was divided by the AUC of the control group) are listed in the Table.

### *2.2. Meta-analysis*

In all publications, AUC values of felodipine were increased following concomitant administration of GFJ compared with that of the control groups. Results of the Q test detected nonuniformity

**Table: Reported pharmacokinetic interactions of felodipine following consumption of GFJ in humans**

| | | GFJ-administration | | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Publication year | Author | n | Dose (mg) | GFJ[a] (mL) | AUC in leterature | AUC/dose (nmol·h/L/mg dose) | n | Dose (mg) | AUC in leterature | AUC/dose (nmol·h/L/mg dose) | AUC ratio[b] |
| 1991 | Bailey et al. | 6 | 5 | 500 | 103.0 ± 367 (nmol·h/L) | 20.60 ± 7.35 | 6 | 5 | 41.0 ± 19.6 (nmol·h/L) | 8.20 ± 3.92 | 2.51 |
| 1992 | Edgar et al. | 9 | 5 | 200 | 65.0 ± 26.3 (nmol·h/L) | 13.00 ± 5.26 | 9 | 5 | 22.8 ± 10.6 (nmol·h/L) | 4.56 ± 2.12 | 2.85 |
| 1993 | Bailey et al. | 9 | 5 | 200 | 41.0 ± 18.0 (nmol·h/L) | 8.20 ± 3.60 | 9 | 5 | 22.0 ± 12.0 (nmol·h/L) | 4.40 ± 2.40 | 1.98 |
| 1995 | Bailey et al. | 12 | 10 | 250 | 56.6 ± 21.9 (ng·h/ml) | 14.70 ± 5.70 | 12 | 10 | 28.1 ± 11.5 (ng·h/ml) | 7.31 ± 2.99 | 2.01 |
| 1995 | Lundahl et al. | 9 | 10 | 200 | 145.9 ± 30.3 (nmol·h/L) | 14.60 ± 3.03 | 9 | 10 | 101.7 ± 21.2 (nmol·h/L) | 10.17 ± 2.12 | 1.43 |
| 1996 | Bailey et al. | 12 | 10 | 250 | 49.7 ± 14.5 (ng·h/ml) | 12.90 ± 3.79 | 12 | 10 | 25.8 ± 11.8 (ng·h/ml) | 6.71 ± 3.07 | 1.93 |
| 1997 | Lown et al. | 10 | 10 | 237 | 76.4 ± 15 6 (nmol·h/L) | 7.60 ± 1.56 | 10 | 10 | 35.3 ± 21.6 (nmol·h/L) | 3.53 ± 2.16 | 2.18 |
| 1997 | Lundahl et al. | 12 | 10 | 150 | 115.0 ± 35.3 (n.mol·h/L) | 11.50 ± 3.53 | 12 | 10 | 66.8 ± 20.B (nmol·h/L) | 6.68 ± 2.08 | 1.72 |
| 1998 | Bailey et al. | 12 | 10 | 250 | 130 ± 52.0 (nmol·h/L) | 13.00 ± 5.20 | 12 | 10 | 53.0 ± 24.3 (nmol·h/L) | 5.30 ± 2.42 | 2.45 |
| 2000 | Bailey et al. | 12 | 10 | 250 | 54.0 ± 27 7 (nmol·h/L) | 5.40 ± 2.77 | 12 | 10 | 25.0 ± 17.3 (nmol·h/L) | 2.50 ± 1.73 | 2 16 |
| 2003 | Bailey et al. | 8 | 10 | 250 | 65.0 ± 31 1 (nmol·h/L) | 6.50 ± 3.11 | 8 | 10 | 36.0 ± 22.6 (nmol·h/L) | 3.60 ± 2.26 | 1.81 |
| 2004 | Goosen et al. | 11 | 5 | 250 | 46.0 ± 12.8 (nmol·h/L) | 9.20 ± 2.56 | 11 | 5 | 34.1 ± 10.2 (nmol·h/L) | 6.82 ± 2.04 | 1.35 |

[a] Volume of double strength GFJ was convert to that of single strength GFJ

[b] AUC ratio=$AUC_{GFJ}/AUC_{control}$

among the data of publications ($\chi^2 = 26.8$, $p = 0.005$), therefore, a random effect model was adopted to integrate the data in a meta-analysis. A forest plot of the analysis is shown in Fig. 1. The total number of subjects was 122. Integrated weighted mean difference (WMD) was 4.38, and the 95% confidence intervals (CI) ranged from 3.67 to 5.10. These results suggest that GFJ administration increases felodipine AUC significantly.

### *2.3. Publication bias*

The funnel plot with a proximal standard error (SE) of the average difference (AD) between AUC values of the GFJ group and the control group in each study is shown in Fig. 2. Publication bias in the studies was strongly suspected because of the left-right asymmetry in the plot. Furthermore, rank correlation analysis (Begg's test) also showed incontestable publication bias with a 0.636 Kendall's $\tau$ rank correlation coefficient ($p = 0.004$). Felodipine is one of the first drugs found to show pharmacokinetic interactions with GFJ following concomitant consumption (Bailey et al. 1989, 1991). In a clinical study Bailey and coworkers reported that the interaction between felodipine and GFJ caused a 2.50 times increase in AUC (Bailey et al. 1991). Subsequent clinical studies on the potential of GFJ interaction with at least 11 dihydropyridine drugs, amlodipine, azelnidipine, benidipine, cilnidipine, efonidipine, manidipine, nicardipine, nimodipine, nisoldipine, nitrendipine, and pranidipine, as well as nifedipine and felodipine have provided further evidence in humans (Bailey et al. 1991, 1993b; Soons et al. 1991; Josefsson et al. 1996; Sugawara 1996; Hashimoto et al. 1998; Fuhr et al. 1998; Uno et al. 2000; Yajima et al. 2003; Hirashima et al. 2006; Ohnishi et al. 2006), anti-malarial agents (van Agtmael et al. 1999a, b), benzodiazepines sedative-hypnotic agents (Hukkinen et al. 1995; Kupferschmidt et al. 1995; Christensen et al. 2002), and antianxiety agents (Lilja et al. 1998; Lee et al. 1999). Among the dihydropyridine drugs, felodipine has been recognized as one with a relatively high risk for interaction. Therefore, it has been used as a positive control in many clinical studies. For example, Goosen et al. (2004) demonstrated that bergamottin, a major furanocoumarin derivative in GFJ, contributed in part to the interaction with felodipine as an interaction-related medication. Lown et al. (1997) came to a conclusion on the relationship between elimination of CYP3A protein in intestinal mucosal cells and the interaction from their investigation using felodipine. On the other hand, the extent of increase in AUC due to the interaction differs greatly among clinical studies. For instance, the maximal and minimal AUC-increasing ratios calculated from plasma felodipine concentrations between the GFJ group and control group were 2.85 times and 1.35 times, respectively (Edgar et al. 1992; Goosen et al. 2004). Such a difference can depend on the concentrations of furanocoumarin derivatives present in GFJ that were used in the studies (Uesawa 2008; Uesawa and Mohri 2008a) and the individual differences in levels of expression of intestinal CYP3A among the subjects (Lown et al. 1994). We attempted to characterize GFJ interaction by consolidating varying clinical researches. Apparently, the forest plot made from the meta-analysis seemed to suggest significant interaction with WMD based on effect size and 95% CI. In the next step of the analysis, the funnel plot was constructed to validate publication bias. In Fig. 2, the average difference in felodipine AUC values (AD) between the GFJ group and control group in each study was plotted on the horizontal axis as a parameter indicating effect size of GFJ. In the plot, reciprocal SE on the mean difference (1/SE) as a parameter related with reliability of the studies was applicable on the vertical axis. The funnel plot was a reversed-funnel shape with the maximal reliability being the point of "true

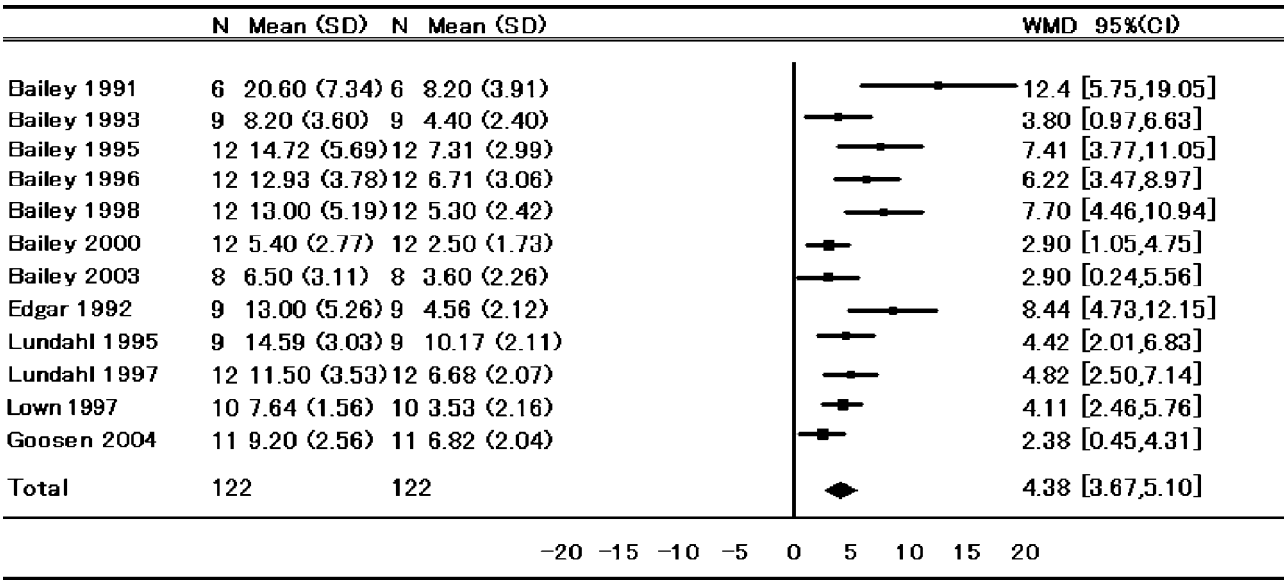


Fig. 1: Forest plot of the average difference of felodipine AUC (nmol.h/L/mg dose) and the corresponding 95% CI from the studies included in the meta-analysis of GFJ-interactions. WMD and the 95% CI were calculated by the general variance-based method

value" in the AUC difference, and is obtainable from a hypothetical large-scale clinical study if there is no publication bias. On the other hand, if the plot is an unsymmetrical shape, bias could be suspected. In the present plot, considerable left-right asymmetry was observed. That is, ADs on the great values of the reciprocal SE slanted leftward in the funnel plot. This result suggests the presence of publication bias and overestimation of the effect of GFJ interaction. The consideration was clearly confirmed statistically by Begg's test. The fact that other drugs possessed the potential for GFJ interaction might also indicate a similar tendency of publication bias in the fundamental clinical studies. More careful estimation of the clinical effects of intake of GFJ on medicines is necessary.

### *2.4. Conclusions*

A meta-analysis for pharmacokinetic interactions between felodipine and GFJ were performed. Integration of AUC values in 12 clinical studies that were selected for this study revealed apparently significant interactions with increase in AUC values in the GFJ groups compared with the control groups. However, a funnel plot indicated the presence of strong publication bias among the studies. These findings suggest that careful attention is required when estimating the clinical impact of medicines related with GFJ interactions in the fundamental stage of clinical studies.

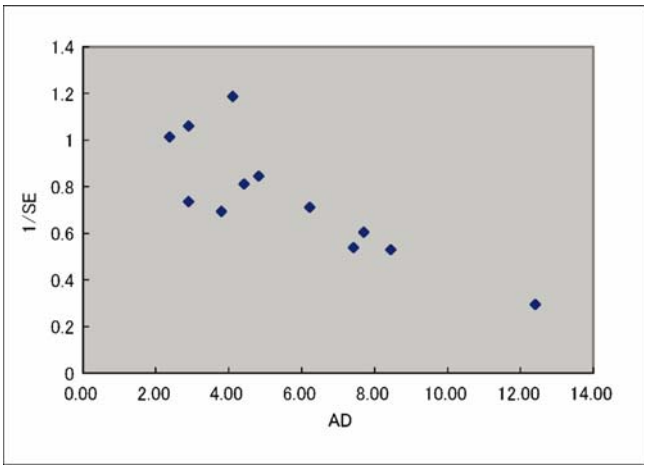


Fig. 2: Funnel plot for increase in AUC (nmol.h/L/mg dose) following GFJ consumption. AD indicates the average difference between AUC in the GFJ group and the control of each study. SE indicates the standard error of AD

## 3. Experimental

### *3.1. Database*

Publications of felodipine-grapefruit juice (GFJ) interactions were searched in Medline (1966 to 2008), Japana Centra Revuo Medicina (from 1983 to 2008) and the Cochrane library (from 1992 to 2007). Furthermore, an additional hand search was performed.

### *3.2. Criteria*

Publications collected in this research met the following criteria; subjects were healthy adults, the study design was a controlled study, felodipine was administrated orally with GFJ at the same time or within one hour after intake of the juice, area under the plasma concentration-time curve (AUC) was included as the endpoint of the interactions, language was not limited, and the control groups administered water in place of GFJ. Independence of the publications selected in the present study was checked based on the contents of the literature such as establishment, volume of GFJ administered, felodipine dosage, and AUC values. These operations were performed by 3 co-workers.

### *3.3. Sampled data*

Publication year, number of subjects, dosage amount of felodipine, volume of GFJ administered, AUC values and standard deviations in the groups that administered GFJ and the controls were collected from publications and are summarized in Table.

### *3.4. Statistics analysis*

The meta-analysis was performed using Cochrane Review Manager 4.2.10 (The Nordic Cochrane Centre, Copenhagen, Denmark). AUC was incorporated into the analyses. AUC values in the literatures were divided by the dose of felodipine in each study, and then the units were arranged by nmol·h/L/mg. Nonuniformity of the publications selected in the previous procedure was checked by Q test. If null hypothesis of uniformity was rejected ($p < 0.05$) in the test, then a random effect model was adopted to integrate the publications in the meta-analysis. On the other hand, if the hypothesis was not rejected ($p \geq 0.05$), a fixed effect model was selected. Statistical significance ($p < 0.05$) of AUC-differences between the GFJ group and the control group was evaluated based on weighted mean difference (WMD), the standard deviation (SD) and corresponding 95% confidence intervals (CI) of the AUC values in each study. Funnel plot was rendered with the AUC-differences on the horizontal axis and reciprocal standard error on the vertical axis. Statistic evaluation of publication bias in the studies was performed by the rank correlation analysis (Begg's method) of funnel plot using an Excel program.

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