VARIATIONS IN CONTENT OF ACTIVE INGREDIENTS CAUSING DRUG INTERACTIONS IN GRAPEFRUIT JUICE PRODUCTS SOLD IN CALIFORNIA

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SUMMARY

The content of the active ingredients of grapefruit juice, naringin, naringenin and bergapten, was determined in 20 different commercial products of grapefruit juice sold in California. These included Minute Maid, Dole, Tropicana, Ocean Spray, Ralps, Albertson, Stater Bros, Vons, Langers, etc. The concentrations of naringin, naringenin and bergapten in grapefruit juice were assayed by specific HPLC methods. Naringin was found to be the most abundant flavonoid in grapefruit juice products, followed by naringenin and bergapten. The content of naringin varied among the products, ranging from 104 mg/l (Tropicana ruby red) to 628 mg/l (Ralphs white frozen concentrate). The mean contents of naringin in ruby red (158 ± 66 [SD] mg/l) and pink (279 ± 123 mg/l) grapefruit juice products were significantly lower than white (481 ± 94 mg/l) ($p < 0.005$) grapefruit juice products. Content of naringenin also varied from brand to brand and ranged from 3.9 mg/l (Vons white frozen concentrate) to 31.2 mg/l (Tree Sweet pink). Bergapten content was very low in grapefruit juice products ranging from 0 (not detectable) to 5.5 mg/l. There were no

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significant differences in naringenin and bergapten contents among the three types of grapefruit juice products. The information gained from this study would be useful in predicting the likelihood of grapefruit juice-drug interactions.

KEY WORDS

grapefruit juice, drug interactions, active ingredients, naringin, naringenin, bergapten, California

INTRODUCTION

Drug interactions with grapefruit juice (GFJ) have been reported for the past few years. GFJ has been shown to increase absorption and the oral bioavailability (2-15 fold) of a wide variety of drugs, e.g., felodipine, cyclosporin, midazolam, triazolam, lovastatin and atorvastatin (see /1/ and references therein). All these drugs are metabolized by cytochrome P450 3A4 (CYP3A4) and have low oral bioavailability as they undergo extensive first-pass metabolism. The mechanism of drug interaction with GFJ is thought to be due primarily to GFJ-mediated inhibition of intestinal CYP3A4 activity without apparent inhibition of hepatic CYP3A4 activity /1/. Inhibition of CYP3A4 by GFJ appears to involve irreversible inactivation of CYP3A4, as evidenced by downregulation of intestinal CYP3A4 protein content without alteration of intestinal mRNA levels /1,2/. This mechanism is likely to be due to mechanism-based inactivation of intestinal CYP3A4, which ultimately results in protein degradation /3/. Further evidence suggests that GFJ might also inhibit P-glycoprotein, but clinical support is still controversial /4-7/.

Much effort has been focused on the identification of the active ingredients responsible for the effect of GFJ. Such knowledge not only would allow the prediction of other foods that might produce this type of food-drug interaction but may also have commercial applications /7/. For instance, by use of separation techniques or genetic engineering, it may be possible to remove the active ingredients from the juice to avoid drug interaction potential. In addition, it may be possible to incorporate purified forms of these components into drug formulations of certain CYP3A4 substrates to improve oral bioavailability and to

reduce the interindividual and intra-individual variation in oral bioavailability. Naringin, the predominant flavonoid in GFJ, and its aglycone, naringenin, were initially suggested to be potential inhibitors of CYP3A4. However, further studies found that furanocoumarin derivatives contained in grapefruit, including bergapten, 6′,7′-dihydroxyberamottin (DHB) and furanocoumarin dimers, had a strong inhibitory effect on CYP3A4 /7-10/. Thus, the amount of flavonoids and furanocoumarins ingested may become an important factor in determining the overall GFJ and drug interactions. The present study was conducted to determine the content of naringin, naringenin and bergapten in different brands of GFJ products sold in California. The study was proposed to test the hypothesis that the content of active ingredients (flavonoids and furanocoumarin compounds) found in grapefruit juice products that cause drug interactions varies between brands. This is based on results obtained from previous studies that were carried out with products sold in New Zealand and Japan /10,11/.

MATERIALS AND METHODS

Naringin, naringenin, bergapten, rutin and 8-methoxypsoralen were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Twenty commercially available GFJ products (n = 3 for each) were randomly purchased from grocery stores in California, USA, during the year 2004. The seven white GFJ products purchased were Ocean Spray, Albertsons, Vons frozen concentrate, Albertsons frozen concentrate, Stater Bros, Minute Maid frozen concentrate and Ralphs frozen concentrate. Five brands of pink GFJ were tested, including Albertsons, Ocean Spray, Vons frozen concentrate, Tree Sweet and Tropicana. The eight ruby red GFJ products were Ocean Spray, Albertsons, Ralphs, Spring Field, Dole, Langers and Tropicana.

Sample preparation

Pure compounds of naringin, naringenin and bergapten were dissolved and diluted in orange juice to make standard solutions for each compound. These standard solutions were used to construct the calibration curves. They were assayed by the same procedures as the samples of GFJ. To determine the content of active ingredients, three samples from each brand of grapefruit juice product were analyzed in

duplicate. To 100 µl of GFJ sample or standard solution, 100 µl of cold methanol containing internal standard was added. Rutin and 8-methoxypsoralen were used as internal standards for naringin and naringenin/bergapten assays, respectively. After vortexing, samples were centrifuged at 1,500 *g* for 10 min, and 70 µl of supernatant was injected into the HPLC column.

HPLC assays

The concentration of naringin in grapefruit juice was assayed by a reversed-phase HPLC method as previously described /12/. The concentrations of naringinen and bergapten in grapefruit juice were simultaneously determined by a HPLC assay /10/. The intra-day assay variation was low with a coefficient of variation (CV) <5%. Standard curves for both naringenin and bergapten were also linear, with intra-day CV less than 7%. The lowest detection limits for naringin, naringenin, and bergapten were 5 mg/l, 2 mg/l and 0.1 mg/l, respectively.

Statistical analysis

The results were expressed as mean values and standard deviations (SD). To assess statistical differences, analysis of variance (ANOVA) followed by a Tukey test for multiple comparison of means was used. A p value <0.05 was considered statistically significant.

RESULTS

Table 1 summarizes the content of naringin, naringenin and bergapten in 20 commercial GFJ products sold in California. Naringin was found to be the most abundant flavonoid followed by naringenin and bergapten. The mean (± SD) naringin content in 20 products tested was 301 ± 168 mg/l. There was a large variation (6-fold) in naringin content between various brands, ranging from 104 mg/l in Tropicana ruby red to 628 mg/l in Ralphs white frozen concentrate. The naringin content was generally high in white GFJ products. The mean contents of naringin in pink (279 ± 123 mg/l) and ruby red (158 ± 66 mg/l) GFJ products were significantly lower (p <0.005) than in the white products (481 ± 94 mg/l), as illustrated in Figure 1. The

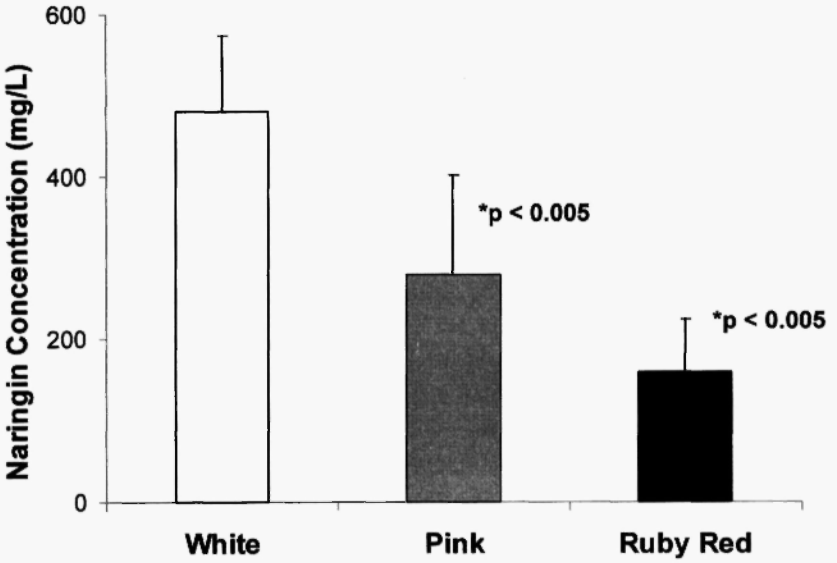

Fig. 1: Content of naringin shown as mean and SD in three types of grapefruit juice products, white (n = 7), pink (n = 5) and ruby red (n = 8). The p values indicate the significant difference between that particular product and the white grapefruit juice products. There was no significant difference in naringin content between pink and ruby red grapefruit juice products.

content of naringin in ruby red GFJ products was slightly lower than that in the pink GFJ, but this difference was not statistically significant ($p = 0.083$).

Naringenin was present at lower concentration than naringin. Overall mean (and SD) content of naringenin in the 20 products tested was 13.5 ± 9.2 mg/l. The content of naringenin also varied widely between the various brands of products (Table 1), ranging from 3.9 mg/l for Vons white frozen concentrate to 31.2 mg/l for Tree Sweet pink GFJ. This represents an 8-fold variation in the content of naringenin. The mean contents of naringenin in white, pink and ruby red GFJ products were 15.4 ± 11.3 mg/l, 14.1 ± 10.2 mg/l and 11.5 ± 7.4 mg/l, respectively. These contents were not significantly different among the three types of GFJ products. The GFJ products contained

TABLE 1

Content of naringin, naringenin and bergapten in different brands of grapefruit juice sold in California

Grapefruit juice product	Type	Concentration (mg/l) [a]		
		Naringin	Naringenin	Bergapten
Ralphs (frozen concentrate)	White	628	9.1	0.4
Minute Maid (frozen concentrate)	White	534	6.7	4.1
Stater Bros.	White	509	25.9	0.2
Albertsons (frozen concentrate)	White	504	6.3	0.5
Vons (frozen concentrate)	White	447	3.9	0.6
Albertsons	White	369	30.0	5.5
Ocean Spray	White	367	25.8	0.4
Tree Sweet	Pink	399	31.2	0.4
Vons (frozen concentrate)	Pink	392	5.4	0.7
Ocean Spray	Pink	282	11.2	3.2
Albertsons	Pink	211	14.8	0.3
Tropicana	Pink	110	7.7	0.7

Langers	Ruby Red	274	28.5	0.8
Florida Natural	Ruby Red	239	5.6	0.2
Dole	Ruby Red	178	7.0	3.8
Spring Field	Ruby Red	142	10.2	0.1
Albertsons	Ruby Red	121	12.4	0.2
Ralphs	Ruby Red	106	10.8	N/D
Ocean Spray	Ruby Red	105	5.7	0.14
Tropicana	Ruby Red	104	12.1	1.3

[a] Results are given as mean of three samples purchased from the same grocery store (i.e. same lot); each sample was assayed in duplicate.
N/D = not detectable, i.e. <0.1 mg/L for bergapten.

very low concentration of bergapten with a mean value of 1.2 ± 1.6 mg/l in all 20 products tested. The content of bergapten ranged from not detectable (<0.1 mg/l) in Ralphs ruby red to 5.5 mg/l in Albertsons white GFJ (Table 1). The mean contents of bergapten were 1.7 ± 2.2 mg/l in white GFJ, 1.1 ± 1.2 mg/l in pink GFJ and 0.82 ± 1.3 mg/l in ruby red GFJ. The bergapten contents were not significantly different among white, pink and ruby red GFJ products. There were no significant correlations between content of these three active ingredients in the GFJ products tested.

DISCUSSION

Taking grapefruit juice with drugs which are metabolized primarily by CYP3A4 has been reported to result in substantial increases in their oral bioavailabilities (see /1/ and references therein). This drug interaction is known to be due to the inhibition of intestinal CYP3A4. The 'bitter principal' of grapefruit juice, naringin, is the most prevalent flavonoid in grapefruit juice and is not present in orange juice. It was previously reported to be present in grapefruit juice at concentrations up to 800-1200 mg/l /10,13/. The aglycone naringenin is found in grapefruit juice at much lower concentrations. After consumption of the juice, naringenin can be formed *in vivo* from naringin, probably in the small intestine /14/. These two grapefruit flavonoids were initially thought to be responsible for the drug interactions with grapefruit juice. However, other grapefruit components, including furocoumarin compounds (e.g. 6′,7′-dihydroxybergamottin, bergamottin and bergapten) and furanocoumarin dimers, may be more important ingredients causing drug interactions /7,8,10/. The contents of naringin, naringenin and bergapten detected in grapefruit juice products sold in California are in agreement with previous studies tested in the products sold in Japan, New Zealand and Mississippi, USA /10,11,15/. The contents of these grapefruit active ingredients varied considerably among different brands of grapefruit juice products. The variation in the content of naringin, naringenin and bergapten found in this study is consistent with those reported previously /10,11,15,16/. Grapefruit juice as a nutrient is not defined with respect to its composition. The contents of active ingredients may vary and such variation may be attributed to differences in fruit maturity, season, and the manufacturing procedure /13/. Although this

study did not determine variation between batches of the same brand of product, other studies have documented batch-to-batch variation in the content of active ingredients in grapefruit juice /10,16/. The rich pink and red colors of grapefruit juice are due to lycopene /17/, a carotenoid phytochemical. So far it is not known whether lycopene may be involved in the drug interactions with grapefruit juice. All products tested in this study appear to be produced from natural grapefruit and were not blended with other fruit juices.

The wide variation of content of the active ingredients (that are responsible for drug interactions) in grapefruit juice products may be important in determining the magnitude of the interaction and whether or not this is likely to be clinically significant. For instance, knowing that bergapten is the most potent CYP3A4 inhibitor as compared to naringin and naringenin /18/, one can predict the likelihood of grapefruit juice drug interactions from its content in the products. With respect to products tested in this study (Table 1), brands with low bergapten content (i.e. Ralphs and Spring Field ruby red) would be unlikely to cause drug interactions, whereas those with high bergapten content, such as Albertsons and Minute Maid white grapefruit juice, would be likely to cause drug interactions. It has been suggested that the large variation of grapefruit active ingredients may also increase the risk of an interaction in patients who drink grapefruit juice regularly and appear to be equilibrated during drug therapy, if the brand is switched during therapy with a potential interacted drug /19/. Further investigations to compare the effect of different brands on the magnitude of the grapefruit juice-drug interaction warrant clarification of this aspect. Variation in content of the grapefruit active ingredients could also explain the contradictory results observed on effects of grapefruit juice on drug pharmacokinetics /14,20,21/. The content of 6′,7′-dihydroxybergamottin (untested in our study because it is not commercially available) is yet another important parameter to be considered. The results from the present study show significant variation in the content of two grapefruit flavonoids and a furanocoumarin (bergapten) in commercial grapefruit juice sold in California. This provides a better understanding of the composition and nature of the active ingredients of grapefruit juice. To minimize prevalent variability in grapefruit juice drug interaction, future studies should consider correlating the study endpoints to the contents of these active ingredients in grapefruit juice products.

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