Review

Inhibition of cytochrome P450 by furanocoumarins in grapefruit juice and herbal medicines

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ABSTRACT

Furanocoumarins (psoralens) exist in various plants and some of them are used to cure skin diseases. These chemicals draw attentions recently because of their abilities to arouse drug interaction through inhibition of cytochrome P450. Grapefruit juice is a well-known example for food-drug interaction. But *in vitro* and *in vivo* studies have shown that the causative components are mainly furanocoumarin derivatives with geranyloxy side chains. *In vitro* experiments confirmed that furanocoumarins from grapefruit juice are both competitive and mechanism-based inhibitors of CYP3A4. Although the inhibition appeared to be stronger in the dimers than that in the monomers, all contribute comprehensively to the grapefruit juice-drug interaction. Further experiments with other furanocoumarins and related *citrus* fruits or *umbelliferous* herbal medicines indicate that drug interaction might also occur with stuffs other than grapefruit juice, especially with traditional medicine.

INTRODUCTION

Furanocoumarins are minor constituents in plants, belonging to *Umbelliferae*, *Rutaceae*, *Moraceae*, and *Leguminosae*. Some naturally occurring methoxy derivatives of furanocoumarins have been applied clinically, coupled with sunbathing, to cure dermatological diseases for more than 2000 years, and their confirmed photosensitizing properties have led to the development of a modern medical principle, photochemotherapy[1]. On the other hand, some plants containing furanocoumarins are also used in the form of crude drugs for traditional medications[2], although it is not clear whether the furanocoumarins are responsible for therapeutic effects.

The biological roles of furanocoumarins in their host plants have not been well understood, but at least these chemicals are considered as natural toxins to protect plants from insects, livestock, and microbes. One of the toxicities of furanocoumarins is related to the inhibition of cytochrome P450s (CYP) that metabolize endogenous and/or xenobiotic compounds. Independent studies using different furanocoumarin derivatives have reported that they can induce or inhibit a wide range of CYP subtypes such as CYP1a1[3], CYP1A2[4], Cyp1b1[3], Cyp2a5[5], CYP2A6[6], CYP2B1[7], CYP6B1/3[8], CYP6B4[9], and CYP6D1[10]. In humans, the well-known grapefruit juice-drug interaction[11] is shown to be caused by some geranyloxy derivatives of furanocoumarins through the inhibition of CYP3A4, a major P450 subtype responsible for drug metabolism in human[12-15].

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In this paper, we will briefly review current understanding on furanocoumarin derivatives from grapefruit juice, and also those from herbal medicines, for their inhibition of human P450 activity as an experimental basis to predict their roles in clinical drug interaction.

CAUSATIVE COMPONENTS IN GRAPEFRUIT JUICES FOR DRUG INTERACTIONS

Grapefruit juice-drug interaction A distinct property of grapefruit juice-drug interactions is that most drugs affected are CYP3A4 substrates undergoing the extensive first-pass metabolism. Thus the drugs are affected only when they are given orally. Considerable data indicate that the effect of grapefruit juice is confined to the inhibition of intestinal CYP3A4. Direct evidences came from Lown *et al*'s *in vivo* experiments[16]: they found that none of the liver CYP3A4 activity, colon levels of CYP3A5, or small bowel concentrations of P-glycoprotein, villin, CYP1A1 was affected after the volunteers had administrated 8 oz (about 227 mL) grapefruit juice 3 times a day for 6 d. The protein level of CYP3A4 in small intestine, however, decreased about one third while the level of CYP3A4 mRNA remained the same. These results suggest that the causative component in grapefruit juice might act mainly in small intestine, and probably as a mechanism-based inactivator of CYP3A4.

Naringin as causative component Soon after the first report of grapefruit juice-drug interaction, it was confirmed that other citrus fruit juice like orange juice had no such interaction, suggesting that grapefruit juice contain specific causative compounds. Naringin is a typical and abundant ingredient in grapefruit juice, and its aglycone naringenin was shown to be an inhibitor of CYP3A4[17], *in vivo* experiments, however, denied it as the responsible candidate[18]. Later, *in vitro* experiments demonstrated that grapefruit juice can directly inhibit microsomal CYP3A activity but the responsible component was neither naringin nor naringenin, which shifted researchers' attention to the extractable fraction of grapefruit juice[19,20].

Discovery of furanocoumarins The extract of grapefruit juice was analyzed independently by several groups. 6',7'-Dihydroxybergamottin (DHB) was reported as the first furanocoumarin for a CYP3A inhibitor in grapefruit juice[12]. Almost at the same period when the American groups focused on furanocoumarin monomers and showed DHB and bergamottin as mechanism-based inactivators of CYP3A4[14,15], Yamazoe's group found two furanocoumarin dimers (GF-I-1 and GF-I-4). These two compounds, being at lower levels in grapefruit juice than bergamottin (GF-I-2), showed CYP3A4 inhibition with potencies as strong as ketokonazole[13]. Fig 1 showed the furanocoumarin derivatives presently known to be existing in grapefruit juice or oil, and with newly assigned names to the dimers[21].
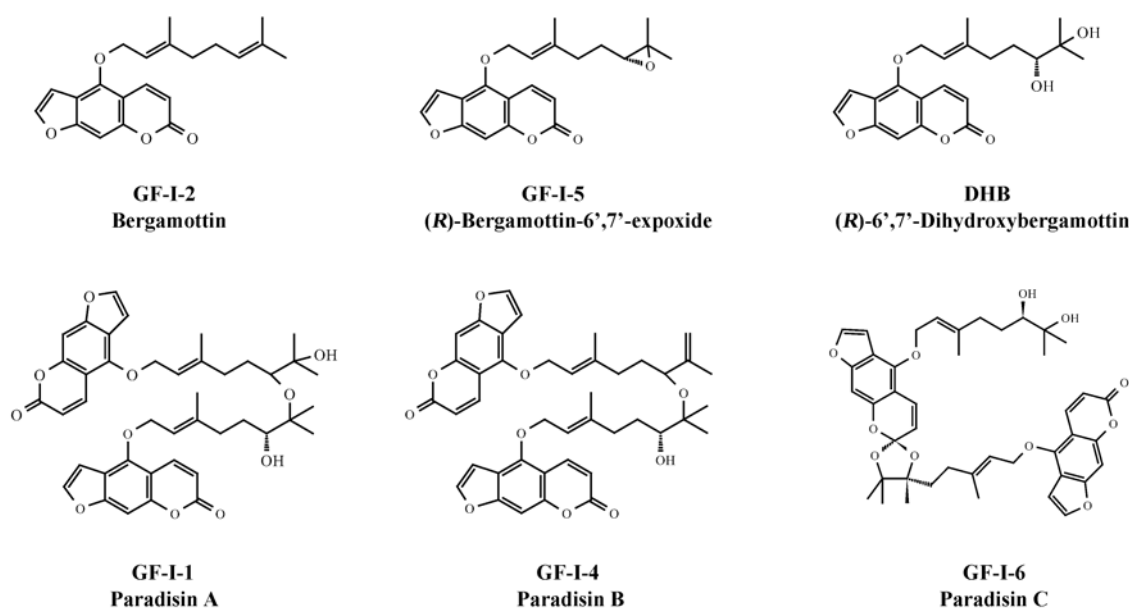

GF-I-2
Bergamottin

GF-I-5
(*R*)-Bergamottin-6',7'-expoxide

DHB
(*R*)-6',7'-Dihydroxybergamottin

GF-I-1
Paradisin A

GF-I-4
Paradisin B

GF-I-6
Paradisin C

Fig 1. Furanocoumarins in grapefruit.

SELECTIVITY AND MECHANISM OF INHIBITION OF FURANOCOUMARINS ON DIFFERENT HUMAN P450 SUBTYPES[22]

Most of the above results were obtained by using testosterone 6β-hydroxylation as a typical rat or human CYP3A activities, but the function of CYP3A has been found to be substrate- or modulator-dependent[23]. So, we used other CYP3A marker activities to confirm the inhibitory effects of furanocoumarins. In addition, the inhibition mechanisms of these furanocoumarins on CYP3A4 have also been investigated separately because reversible and irreversible inhibitions can cause different clinical consequences.

Inhibition of different CYP3A4 activities Our results showed that other CYP3A4-catalyzed reactions (nifedipine oxidation, omeprazole 3-hydroxylation, and omeprazole sulfoxidation) were also inhibited by the main furanocoumarins from grapefruit juice, two monomers (GF-I-2 and DHB) and two dimers (GF-I-1 and GF-I-4). Like the results with testosterone 6β-hydroxylation, dimers showed to be much stronger inhibitors of CYP3A4 activities than did monomers.

Mechanism-based inactivation of CYP3A4 Along with that furanocoumarins showed direct and concentration-dependent inhibition of CYP3A activities when incubated simultaneously, preincubation of furanocoumarins in the reaction mixture before addition of a substrate (nifedipine) caused the profound inhibitory effect. Thus the inhibition depended on the time of preincubation, suggesting the involvement of a mechanism-based inactivation of CYP3A4. K_I values (the inhibitor concentration required for half-maximal rate of inactivation) for GF-I-2, DHB, GF-I-1 and GF-I-4 on nifedipine oxidation in human liver microsomes were calculated as 40.00, 5.56, 0.31, and 0.13 μmol/L, respectively, suggesting that dimers GF-I-1 and GF-I-4 are stronger CYP3A4 inactivators than monomers DHB and GF-I-2. Between the two monomers, our results showed that DHB is more potent than GF-I-2 for CYP3A4 inactivation. Using a different CYP3A4 source with testosterone as a substrate, other researchers found that the potency of inactivation of DHB (K_I 59 μmol/L)[14] was weaker than that of GF-I-2 (K_I 7.7 μmol/L)[15], but their results also confirms that both the monomers are weaker inactivators when comparing their K_I values with those of the two dimers.

Inhibition of other P450 subtypes Couple reports have shown that furanocoumarins inhibit a wide range of P450s in species from insects to mammals as mentioned above, these data suggest the possibility that different P450s in humans may also be affected by furanocoumarins. So, the inhibition of human P450s besides CYP3A4, caused by the furanocoumarin monomers (DHB and GF-I-2) and dimers (GF-I-1 and GF-I-4) isolated from grapefruit juice, were tested by using selective substrate probes.

In contrast to the results from the extract of grapefruit juice which show clear inhibitory properties on microsomal CYP3A4, CYP1A2, CYP2C9, and CYP2D6 with similar extents (IC$_{50}$: 15-28 mg/L), the isolated furanocoumarins had different selectivities for inhibition on human P450s. Apparent selectivity towards CYP3A4 occurred with the furanocoumarin dimers. DHB inhibited CYP3A4, and CYP1A2 activities at nearly equivalent potencies. GF-I-2 showed rather stronger inhibitory effect on CYP1A2, CYP2C9, CYP2C19, and CYP2D6 than on CYP3A4. This result is consistent with a reported study, in which GF-I-2 showed clear inhibition of CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, with stronger potencies on CYP2C9 and CYP2D6[15].

Based on the fact that both grapefruit juice and its furanocoumarin components inhibit human microsomal P450 activities besides CYP3A4, one clinical study was conducted to see whether *in vitro* findings could also happen *in vivo*. When omeprazole was orally taken with grapefruit, the CYP3A4-mediated omeprazole sulphone formation was reduced, but the CYP2C19 mediated 5-hydroxyomeprazole formation was not affected[24], maybe due to the liver-specific existence of CYP2C19 and the confined grapefruit juice reaction within gut wall. Another clinical study on dextromethorphan also showed that its gut CYP3A4, but not liver specific CYP2D6, was affected by grapefruit juice and Seville orange (a species of sour orange) juice[25].

On the other hand, *in vivo* studies have shown that two furanocoumarin drugs, 8-methoxypsoralen and 5-methoxypsoralen were inhibitors of CYP1A2[4, 26] and/ or CYP2A6[6].

SPECIFIC EXISTENCE OF FURANOCOUMARINS IN GRAPEFRUIT JUICE[27]

Soon after the finding that furanocoumarins contribute to grapefruit juice mediated CYP3A inhibition, it was found that DHB, GF-I-2, 6',7'-epoxybergamottin (GF-I-5), and FC726 (possibly GF-I-1) exist in various grapefruit products[14]. We also confirmed that GF-I-1, GF-I-2 and GF-I-4 appear mainly in pulp in compari-

son to sac, peel or seed of grapefruits. The composition of GF-I-1, GF-I-2, and GF-I-4 in grapefruit pulp are similar to those in juice product, excluding the suspect that furanocoumarins might be the byproducts during juice processing. GF-I-1, GF-I-2, and GF-I-4 are not detected in juice samples of apple, grape, orange and tangerine, only trace amounts of GF-I-2 and GF-I-4 appeared in lemon juice, suggesting that furanocoumarins are fairly selective in grapefruit juice.

COMPREHENSIVE ROLES OF FURANOCOU-MARINS IN GRAPEFRUIT JUICE-MEDIATED CYP3A4 INHIBITION[28]

The main furanocoumarins in grapefruit juice reduce CYP3A4 activities through both competitive and mechanism-based inhibition, but the exact role of each compound in the grapefruit juice-drug interactions remains unclear. To verify whether furanocoumarins are the components causing drug interaction, various commercial juice samples from grapefruit and related citrus fruits were tested for both their CYP3A4 inhibition and compositions of furanocoumarins. To obtain a full view on the role of furanocoumarins in grapefruit juice-drug interaction, two newly isolated components, GF-I-5 and GF-I-6 (K_I 37 μmol/L and 23 μmol/L respectively, estimated data, not published), were also investigated together with the previous known components (DHB, GF-I-1, GF-I-2, and GF-I-4). In addition, furanocoumarins isolated from grapefruit juice were tested for their CYP3A4 inhibition separately or in different ways of combination at the concentration levels as they appeared in grapefruit juice.

Experiments with different grapefruit juice samples Among the grapefruit juices from different strains (white or pink), origin (USA, Australia, or Israel), packages (in glass, paper, plastic, or metal container) and ways of processing (reconstituted, straight, or containing 10 % pulp), furanocoumarin compositions determined were similar qualitatively (except for GF-I-6) but differed quantitatively (except of the unstable GF-I-5). When tested at a 2.5 % level, all the juice samples inhibited microsomal CYP3A4 activity to less than 60 % of the controls. The inhibitory potency of grapefruit juice would be expected to correlate with the content of a specific component if one of furanocoumarin derivatives were solely responsible for the inhibition of CYP3A4 activity. The inhibitory potency of grapefruit juice samples tended to be higher with grapefruit juice

containing higher amounts of total furanocoumarins, but showed no clear correlationship with a specific content of any single component.

Experiments with different grapefruit juice fractions After a grapefruit juice (Ki-1) sample was centrifuged and separated into precipitate and supernatant fractions, the precipitate showed inhibition of CYP3A4 activity as strong as that of the original juice sample. The supernatant showed much weaker CYP3A4 inhibition. Consistently, furanocoumarin derivatives, GF-I-1, GF-I-2, GF-I-4, GF-I-5, and GF-I-6 distributed mainly in the precipitate, only DHB localized 2.6-fold more in the supernatant. Two independent studies *in vivo* using different grapefruit juice fractions[29] or grapefruit segments[30] also suggest that DHB is unlike to be the main causative furanocoumarin in the grapefruit juice-felodipine interactions, though its contribution is not denied.

Reconstitution of isolated furanocoumarins To address the role of furanocoumarins, reconstitution of grapefruit juice with furanocoumarins was conducted. When tested with a single component at the highest level observed in juice, none of the furanocoumarins inhibited microsomal CYP3A4 activity to the same extent as the weakest grapefruit juice showed. On the other hand, the combined mixture of furanocoumarins, each at the highest observed level, inhibited CYP3A4 activity as strong as did the strongest grapefruit juice samples. Furthermore, omission of any one of the furanocoumarin compounds (except GF-I-5) from the mixture weakened the inhibitory potency. These results suggest that all the major furanocoumarins are necessary for the maximal inhibition of CYP3A4 activity observed in grapefruit juice. It seems that each furanocoumarin contributes to the grapefruit juice-drug interaction due to either stronger CYP3A4 inhibitory potency (like dimers GF-I-1 and GF-I-4) or higher natural abundance (like DHB and GF-I-2).

Experiments with other citrus fruit juices Taxonomic research showed that cross of pummelo and mandarin produced sweet orange and sour orange, also cross of pummelo and sweet orange produced grapefruit[31]. Cross of pummelo and grapefruit produced sweetie[32]. Among the related citrus fruits investigated, two sweeties, three pummelos and one sour orange, in which furanocoumarins were detectable, showed CYP3A4 inhibition. The extent of inhibition with a sweetie, which contains high levels of furanocoumarins, was stronger than that of grapefruit juice. In contrast,

no clear inhibition was observed with two sweet orange juices in which no furanocoumarin was detected. These results suggest that cautions may not be restricted to grapefruit since pummelo and more than one of its crossed varieties also contain furanocoumarins.

Recently, other groups' clinical studies with Seville orange confirmed the role of furanocoumarins in grapefruit juice-drug interaction. The first one showed that a Seville orange juice, with comparable DHB level to grapefruit juice, did decrease the enterocyte CYP3A4 protein, but could not repeat grapefruit juice-cyclosporine interaction[33]. The second one showed that a Seville orange juice with DHB and GF-I-2 at half level than grapefruit juice, repeated the grapefruit juice-felodipine interaction perfectly, furthermore, another furanocoumarin, bergapten, was found in Seville orange and shown to be a mechanism-based inactivator[34]. Since cyclosporine is a substrate of both CYP3A4 and P-glycoprotein (P-gp, a transporter) whereas felodipine is only a substrate of CYP3A4[35], it is suggested that the *in vivo* role of furanocoumarin components is the inhibition of CYP3A4, but some other component(s) in grapefruit juice may inhibit P-gp.

INHIBITORY EFFECTS OF VARIOUS FURANOCOUMARINS ON HUMAN MICROSOMAL CYP3A4 ACTIVITY[36]

To clarify the relationship between chemical structure and inhibitory potency, furanocoumarins with varying core structures or side chains, as well as some structurally related coumarins, were tested in comparison with their inhibitions of CYP3A4.

Chemical structures of furanocoumarin derivatives examined are divided into 3 groups: 30 monomers, 11 dimers, and 1 trimer. The monomers include two angular ones with furan ring attached to 7,8-position of coumarin, the others are linear ones with furan ring attached to 6,7-position, and substituted with methoxy, prenyloxy or geranyloxy side chains at 5- and/or 8-position. The dimers are two linear furanocoumarin monomers connected by an ether linkage between their side chains or by spiro formation with the side chain of one monomer attached to the pyrone ring of the other monomer. Except for 5 monomers with a geranyloxy side chain and 4 dimers isolated form grapefruit juice or chemically synthesized, all the other furanocoumarin derivatives were isolated from *Umbelliferous* crude drugs such as "Baizhi" (*Angelica dahurica* root), "Hamaudo" (*Angelica japonica* root), "Qianghuo" (*Notopterygium incisum* root), "Fangfeng" (*Saposhnikovia divaricata* root), and "Yunnan Qianghuo" (*Pleurospermum rivulorum* root).

On simultaneous incubation with a substrate in the enzyme reaction mixture, most furanocoumarins (0.1 mmol/L) reduced human liver microsomal testosterone 6β-hydroxylation to less than 50 % of the control. Comparison of their inhibitory potencies showed that their inhibitions on human CYP3A4 activity were as strong as a typical inhibitor, metyrapone (IC$_{50}$: around 10 μmol/L). Particularly, furanocoumarin dimers and a trimer showed strike inhibitions with potencies similar to that of a strong CYP3A4 inhibitor, ketoconazole (IC$_{50}$: around 0.1 μmol/L). Substitution of one of the pyrone ring to form spiro dimers resulted in the reduced inhibition (IC$_{50}$: >0.2 μmol/L) than did the side-chain connected dimers (IC$_{50}$: <0.1 μmol/L). The trimer showed CYP3A4 inhibition similar to the dimers. On the other hand, the results of two angular furanocoumarin monomers and several simple coumarin derivatives with various side chains but lacking a furan ring showed much weaker inhibitory effects (IC$_{50}$: around 100 μmol/L or no inhibition until 100 μmol/L, the data for coumarins were not published). These results suggest that a plain core structure, tricyclic ring containing a furan moiety in linear position, is more important than the side chains for furanocoumarins in CYP3A4 inhibition.

HERBAL MEDICINES AS POSSIBLE CANDIDATES FOR DRUG INTERACTION[37]

Furanocoumarin derivatives have been found in various plants belonging to families like *Rutaceae*, *Umbelliferae*, *Moraceae,* and *Leguminosae*. Many of these plants are source of common beverages, vegetables, or herbal medicines. Besides the daily consumption of beverages like grapefruit juice and vegetables like celery, herbal medicines are often applied for long term therapies, especially the latter is inevitable from coadministration of other drugs. Thus, it is necessary to check whether drug interaction through P450 inhibition will also occur with the use of herbal medicines that contain furanocoumarins.

Traditionally, a prescription of herbal medicine usually consists of several crude drugs, and used in the form of a boiled water decoction or an alcoholic infusion. Therefore, the decoction or infusion of some *umbelliferous* or *citrus* crude drugs and related traditional prescriptions have been investigated for their CYP3A4 in-

hibition and the relationship of inhibitory potencies with their furanocoumarin compositions.

The selection of crude drugs is based on the reason that they contain furano-coumarins and are widely used. Although the samples we collected were not always showing furanocoumarin existence in the form of hot water decoction or 40 % ethanol infusion, the co-variations of furanocoumarin amounts among the samples of crude drugs with their potencies of CYP3A4 inhibition again suggests that furanocoumarins do play an important role in these crude drugs for their inhibitory effects on a drug metabolizing enzyme. Among the samples we collected, a Japanese Baizhi (BZ1, dried root of *Angelica dahurica* BENTH et HOOK var *dahurica* BENTH et HOOK) showed clear relationship between the CYP3A4 inhibition and the furanocoumarin abundance. Another Japanese group also proved that *A. dahurica* extract can inhibit rat microsomal CYP2C, CYP3A, and CYP2D1, and affect the pharmacokinetics of tolbutamide and diazepam in rats[38].

After preincubation, both decoction and infusion of BZ1 showed over three-fold inhibition of CYP3A4 activity. Some other crude drugs and a related prescription also showed increased inhibition after the preincubation, implying that mechanism-based inactivation may also occur in traditional medicine related drug interactions.

On the other hand, some formulated prescriptions showed rather intense CYP3A4 inhibition with their hydrophilic fractions that is unlikely to be related to furanocoumarin. This implies that components other than furanocoumarins in traditional medicines may also be able to inhibit CYP3A4 activity.

PERSPECTIVES

Based on the reports at present stage, it can be concluded that furanocoumarins are the major components for grapefruit juice-drug interactions, especially on small intestinal CYP3A4 inhibition. Although the experiments with Seville orange or any other foodstuff suggest the role of furanocoumarins, the results could not be a decisive one unless otherwise an isolated or chemically synthesized compound reproduced the interaction under similar clinical conditions.

There is a consideration to make the use of grapefruit juice-drug interaction to improve the drug efficacy and to save cost for expensive medicines. It might be feasible if we have fully understood its mechanism of action, and are also able to control the amount and vari-

ance of the causative components. Since there are increased evidence that the causative components differ depending on the drug affected, and under certain conditions, grapefruit juice may also affect hepatic P450[39], it is too impulsive now to apply grapefruit juice into clinical practice.

Some mechanism-based inactivators have abilities to cause induction of P450s. While we and others have shown the inactivation effects of furanocoumarins, a recent report suggested the induction of rat hepatic P450 after long-term administration of grapefruit juice[40]. The amount of furanocoumarins administrated through a cup of grapefruit juice is much less than the dose for 8-methoxypsoralen or 5-methoxypsoralen, two furanocoumarin drugs used in skin diseases. If large amount of those grapefruit juice components become available, their short-term and long-term effects on both intestinal and hepatic P450s would be more clear through various interesting research projects.

Since furanocoumarins are found in a wide range of plants including many common foodstuff, their possible drug interactions are necessary to be clarified. On the other hand, many frequently used herbal medicines also contain furanocoumarins. Their role in drug interaction might be an necessary and important mechanism for the action when they are included in many traditional prescriptions.

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